Neural Mechanisms of Spatial Memory

Dissociating the Encoding and Retrieval of Hippocampal Replay

by

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, MD

August, 2014

Abstract

How memories are encoded and stored is a fundamental question in neuroscience. The hippocampus, a well-established brain region for memory and learning, plays a critical role in the initial encoding and consolidation of spatial memory. The discovery of place cells, hippocampal neurons that increase their firing rate when an animal is in a particular region of an environment, was the first demonstration that space could be encoded in the firing pattern of the hippocampus. The place field, a place cell's preferred firing region, only encodes spatial information about the animal's current location. However, spatial navigation and memory involve a sequence of locations. By analyzing hippocampal spatial activity at the network level, recurring sequences matching the rat's previous spatial experience were found in both sleep and awake behavior. Replay, the sequential reactivation of a recent trajectory played in reverse, occurs during memory retrieval and is important for the establishment of memory traces. Thus, it is of considerable interest to understand how and when spatial replay occurs.

This dissertation examines the hypothesis that hippocampal replay constitutes a neuronal model system for memory formation dependent upon both experience and NMDAR function. We employed ultrahigh density recording methods to monitor hundreds of place cells simultaneously during novel experience, enabling measurement of spatially coordinated activity on timescale of tens of milliseconds, and measurement of the depiction of moving trajectories on a 100ms timescale. We demonstrate that hippocampal replay is dependent on molecular mechanisms associated with learning-

related plasticity and are able to dissociate the encoding and retrieval phases of memory processing during spatial navigation.

Thesis committee: David Foster (advisor), Jim Knierim (chair), David Linden (reader), and Alfredo Kirkwood.

Acknowledgements

It is with immense gratitude that I acknowledge and thank all those who have been a part of my pursuit of knowledge. I begin with my advisor, Dr. David Foster, without whom this work would not exist. I would like to thank him for his ingenuity and enthusiasm. His imagination and creativity have no limits, thereby making every member of his lab feel as though there are no unsurmountable questions. I would also like to thank him for his continued support in all my endeavors, in particular, his unremitting excitement and encouragement throughout all our conversations. He took a gamble on me, trusted me, and allowed me to be independent and truly follow the path that my data took me on.

I would like to thank my thesis committee, Dr. Jim Knierim, Dr. David Linden, and Dr. Alfredo Kirkwood. I chose each committee member because of their level of expertise, and as individuals whom I truly look up to. I would like to thank them all for their time, encouragement, and insightful comments.

I thank all of my labmates. The first 'inhabitants', Dr. Xiaojing Wu and Dr. Brad Pfeiffer, with whom I learned how to set up a lab and established the lab's methodologies. We were a strong trio, who worked very well together. There are many memories that were created after our long days doing surgeries, multiple trips to Home Depot, and multiple troubleshooting days. The lab has since grown, and I would like to acknowledge and thank each one of them. I would like to thank Ting Feng, who worked with me during her rotation, and I tried very hard to get excited about my work. Lucky for me, she joined the lab. Together we have become the D-CPPene duo. She has been a

valuable member of the lab, with whom I have explored multiple theories and analytical processes. She has taken the same set of data presented here, and analyzed the oscillatory events for her own thesis work. I would like to thank, Dr. Cara Altimus, Ellen Ambrose, Christine Boone, Heydar Davoudi, and Jon Harrold for making the Foster lab what it is today, a fun, insightful place to ask intriguing questions about the mechanistics of the brain.

There are other members of the Neuroscience Department that I would like to acknowledge. I would like to thank Dr. Alex Kolodkin, for introducing me to Johns Hopkins Neuroscience and for his continued support throughout my time at Hopkins. I would also like to thank Dr. Dwight Bergles and Dr. Nicholas Gaiano for allowing me to rotate in their labs as I explored different interests within the field of Neuroscience.

On a more personal level, there are a great number of people with whom I interacted that have helped shape my journey in life to where I am today. I could write a whole separate book to include all those people, and so I will refrain from doing so and thank my most immediate family. I have many things to thank my parents for, but one of the most important things is the gift of education, and their continued support and trust. As an only child, only girl, in a small family, born in a small fishing village off the coast of Galicia, Spain, they had big dreams for me well before I could even dream. They had no idea what I was up to most of the time, but they always let me make my own decisions and watched patiently to see where my curiosity led me. My wonderful grandmother and second mom, who just turned 90, always referred to me as the “doctor of rats” and wondered why I spent my weekends in lab instead of searching for a husband. She’s always been a phone call away and it is true what they say, with age, her wise words have

become an integral part of my every day. And my now husband, Nicholas Herbert, for providing me with the emotional support to push through the last years of the PhD, and always helping me keep things in perspective. His infectious positive attitude and optimism make the impossible worth trying.

To my loving parents, for teaching me to never say, “No, I don’t know” and instead ask, “Tell me, how can I learn?”

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Introduction

The subject of memory is one that fascinates many. It is a topic that has intrigued philosophers, authors, and even movie directors for centuries. Memory is a part of our every day activities. In a present moment, we remember what we did yesterday, use our knowledge that we may have acquired a decade ago, and we learn new things and create new memories, which we may use tomorrow. In this dissertation, I look at spatial memory to try and understand the processing necessary for encoding and retrieving memories. I focused on spatial memory in the rat because it is much more feasible to experimentally ask an animal "where have you been?" and evaluate the result, than to ask "what are you thinking?" It is also important to note that although there have been a great number of technological advances in the field of neuroscience, the ability to probe the human brain in a manner allowing us to look at single cells as we do in the work described here remains to be a challenging undertaking.

The hippocampus, a structure in the medial temporal lobe, plays a critical role in memory as shown by the fact that damage to the hippocampus leads to severe amnesia in particular for episodic memory and navigational memory. (Gaffan and Harrison, 1989; Morris et al., 1982; Olton and Samuelson, 1976a; Olton and Samuelson, 1976b; Scoville and Milner, 1957). Episodic memory is the term that has been used to define memories of a unique event or temporal sequence of events that comprise an episode (Tulving, 1983). It is also thought of as the mental representation of the "what", "when", and "where"; and is best described as a memory of the past requiring mental travel that is encoded automatically as it is being experienced, and includes the time and place of the

event (Tulving, 2002). Although many types of memories and terminology have been defined, an understanding of how neurons in the hippocampus support memory has remained elusive.

Single-unit recordings in awake and behaving rats(O'Keefe and Dostrovsky, 1971), mice(McHugh et al., 1996), monkeys(Matsumura et al., 1999) and people(Ekstrom et al., 2003) have revealed that hippocampal neurons exhibit spatial receptive fields during the exploration of an environment. These pyramidal cells were termed place cells and the location of their firing, place fields. They were first discovered by O'Keefe and Dostrovsky in 1971, when they recorded from CA1 cells during different behavioral tasks and different sensory stimulations and noted that the activity of these cells was associated to the animal's location and not other aspects.

Even though the hippocampus is critical for the encoding of spatial memory, there is no topographic map in the hippocampus. Locations are represented by a distributed population code, with anatomically distant cells having overlapping fields. Place cells create an ensemble of place fields that cover the entire environment. As an animal moves from one location to another, the sequence of place fields crossed represent the animal's path. The location of a place field in one environment yields no information about its field in a different environment, nor do its neighboring anatomic connections. Still, hippocampal pyramidal cells can represent many environments without interference since only about half of the 10^6 pyramidal cells are place cells, or fire, in a given environment(Tanila, 1999). Interestingly, the stability of place fields in a familiar environment suggests that place cells encode long-term memory of that

environment(Kentros et al., 1998). Remapping, the change in place cell representation observed when a familiar environment changes, further validates this suggestion.

Place cells are considered "memory cells" because they can provide the "when" and "where" of an episodic memory. However, the relationship between place cell activity and memory is not immediately clear. A persistent mystery has been how activity that is temporally and spatially localized might relate to memory or navigation. Place cells might tell an animal about where it is, but not about where it's been or where it's going. Furthermore, memory for events is frequently separated into encoding and retrieval phases(Morris et al., 2003). Receptive field responses do not map onto these separate phases in an obvious way.

What remains to be answered is how the neuronal activity is translated into memory. In addition to firing in their place fields, place cells exhibit offline activity known as replay, in which cells fire in precise sequences corresponding to previous experiences(Davidson et al., 2009; Diba and Buzsaki, 2007; Foster and Wilson, 2006; Gupta et al., 2010; Karlsson and Frank, 2009; Lee and Wilson, 2002; Louie and Wilson, 2001). Replay sequences occur in a temporally compressed format, and, except for the case of REM sleep, are synchronous with sharp-wave ripple (SWR) events in the hippocampal local field potential. Recent work has demonstrated that in a spatial memory task, SWR-associated place-cell sequences depict immediate future behavioral trajectories to a remembered goal location (Pfeiffer and Foster, 2013). Thus, offline place-cell sequences have the capacity to address the mnemonic shortcomings of place field responses, since they can depict memories of past behaviors, and do so selectively in response to the current demands of a memory task. Moreover, replay sequences offer a

straightforward separation into encoding (during experience) and retrieval (during replay) phases.

Place cell responses are observed immediately upon the first experience in an environment (Epsztein et al., 2011; Hill et al., 1978). Replay is observed during stopping points immediately after the running behavior in an environment and in sleep (Carr et al., 2011). Repeated exposures to an environment or to multiple environments can affect single unit activity. How ensemble activity or the generated place field map changes with repeated exposures is not very well characterized. Even less understood is the sequence of active cells, or replays, that are generated during sleep and stopping periods. In this dissertation, I investigate place cell activity in the same animal during awake behavior and in sleep or quiet rest. We attempt to dissociate memory and experience at the neuronal level, thereby providing an association between spiking activity observed during the running experience and during memory formation.

The most striking difficulty in relating place cell activity to memory concerns the plasticity mechanisms that have long been thought to underlie learning and memory. Blockade of N-methyl-D-aspartate receptors (NMDARs) that are critical for activity-dependent, associative and long-lasting plasticity throughout the brain, prevents spatial learning and event memory under a wide variety of conditions (Caramanos and Shapiro, 1994; Morris, 1989; Morris et al., 1986; Shapiro and Caramanos, 1990; Steele and Morris, 1999). A particularly striking feature of these results is that while encoding of new memories is blocked under NMDAR blockade, retrieval of previously formed memories is not(Bannerman et al., 1995; Morris, 1989; Saucier and Cain, 1995). However, under NMDAR blockade, hippocampal neurons exhibit apparently normal

place fields(Kentros et al., 1998). Where effects have been reported, they tend to be subtle such as changes in place field size(McHugh et al., 1996), or changes to place fields due to cue manipulations(Nakazawa et al., 2002) that are not necessary in order to observe memory deficits. Taken together, these studies suggest that the existence of neurons with spatially restricted place fields is not a sufficient condition for hippocampally dependent learning and memory. This in turn suggests that some further property of hippocampal neural activity must support the hippocampal role in learning and memory.

In this dissertation, we hypothesize that hippocampal replay constitutes a neuronal model system for memory formation dependent upon NMDAR function. We propose that if place-cell replay sequences are a critical mechanism of hippocampally dependent memory, then replay should fail to occur for behavioral sequences experienced under NMDAR blockade, and, by analogy with hippocampus dependent behavioral memory, that replay might become independent of NMDARs once memory has already been established. We begin with an introductory Chapter 1, which describes the founding work that led to the discovery of the role of the hippocampus in spatial navigation and memory formation. In the chapters that follow, we describe the work that we performed to understand the neural mechanisms behind the different phases of spatial learning and memory. In Chapter 2, we focus on our analysis of place fields under NMDAR antagonism and address stability and selectivity as we characterize place cell firing in multiple novel environments. We introduce the experimental design and methodologies applied in Chapter 2 and continue to further describe our analytical methods in Chapter 3. We define replay in Chapter 3 and explore how experience and NMDAR antagonism

affects the encoding of replays. We identify two distinct forms of temporal structure and find that under NMDAR blockade replay sequences encoding a trajectory for a novel environment are abolished. In Chapter 4, we look at the retrieval of replays and address the persistence of memory traces. The final chapter describes the future research that will build upon the findings discussed in this dissertation, some which offer contradictory evidence to early proposed theories and will require re-examination and further analysis.

Chapter 1: The Hippocampus

Introduction

The hippocampus is found deep in the medial temporal lobe, and is most studied for its role in memory. It was anatomist Julius Caesar Aranzi (1587), who first compared the shape of the hippocampus to that of a seahorse. "Ram's horn" was coined by the Danish anatomist Jacob Winsløw in 1732; and a Parisian surgeon de Garengeot, used "cornu Ammonis" meaning horn of Amun, the Egyptian god. The different terminology captured the curvature of this brain structure that lies underneath the cortex, extending caudally between the neocortex and diencephalon before curving ventrally toward the temporal lobe (Witter et al., 2004). The hippocampus itself is divided into two major U-shaped interlocking sections, the fascia dentata, the dentate gyrus, and the hippocampus proper, cornu ammonis, CA1, CA2, CA3, and CA4 (O'Keefe and Nadel, 1978). The hippocampus can be further divided by cell morphology and internal fibre projections.

The different neuronal cell types are neatly organized into layers in the hippocampus, making the hippocampus the model system for studying neurophysiology. It is the densely packed pyramidal neurons that form the molecular layer that lend themselves experimentally for electrophysiological recordings which gave way to the early the discovery of waves of patterns of neural population activity. In 1938 Richard Jung and Alois Kornmüller noted desynchronization of the neocortical electroencephalogram, EEG, was temporally linked to large-amplitude, sinusoidal wave patterns in the rabbit hippocampus between 4 and 7 Hz, termed 'theta' activity (Wilmer

et al, 2004). This was thought to be related to enhanced attention and it was proposed that theta activity could be coupled to specific learning states (Grastyan et al., 1959).

Many theories about the function of the hippocampus came well before functional studies could be assayed. Until the 1930s the hippocampal formation was thought to be part of the olfactory system. In 1937, James Papez suggested the hippocampus was part of a circuit that provides the anatomical substrate of emotion. The early theories of hippocampal function were derived from anatomical studies, soon to be overtaken by lesion studies in both animals and humans and advanced behavioral animal models that have led to the current extensive literature about the hippocampus's role in learning and memory, and in spatial navigation.

Hippocampal Anatomy

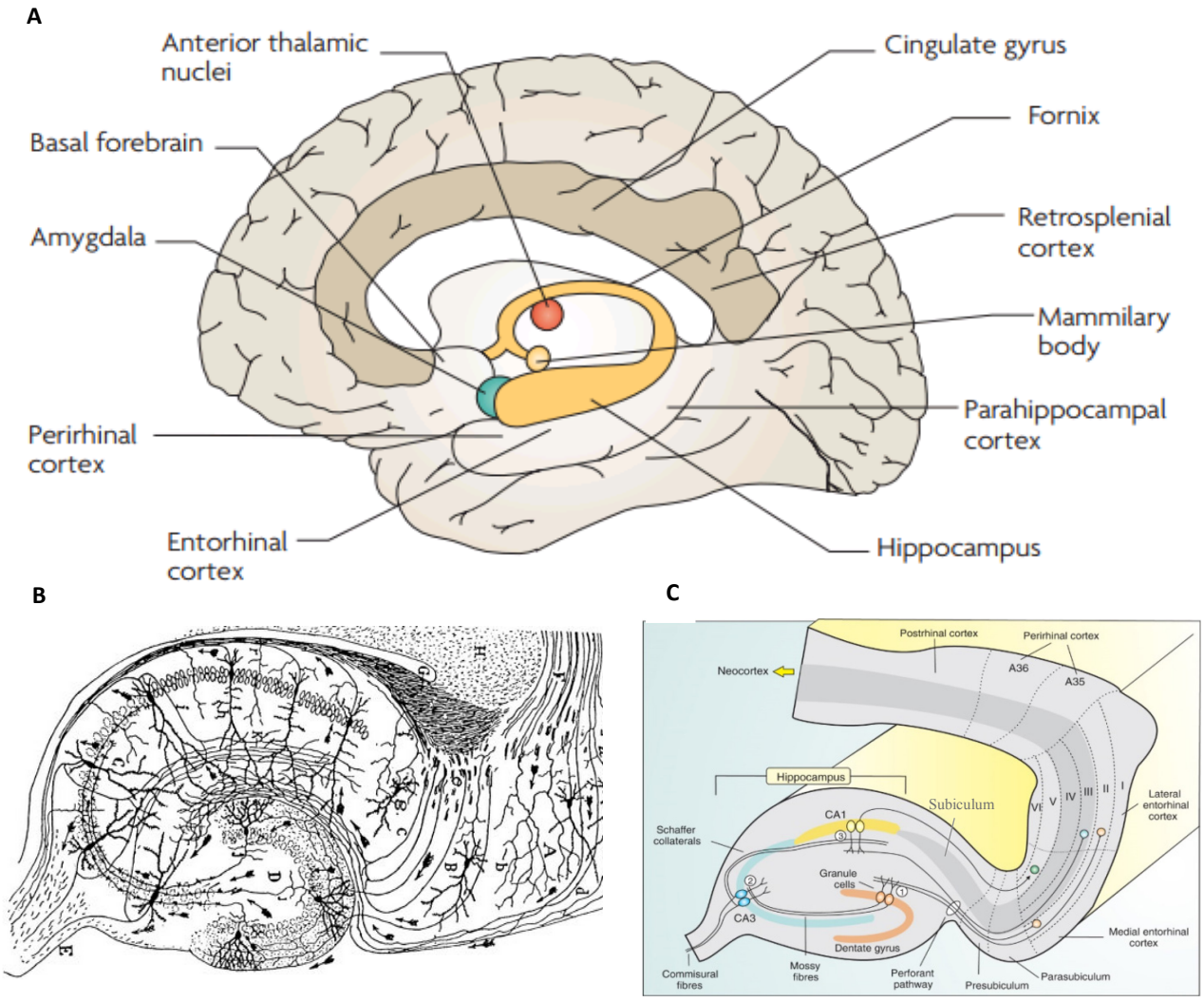


Figure 1. Hippocampal Circuitry. A, The hippocampus lies in the medial temporal lobes, surrounded by the entorhinal, parahippocampal and perirhinal cortices (Bird and Burgess, 2008). B, Ramon y Cajal's rendition of the neural circuitry in the hippocampus (Cajal, 1911). C, The organization of the circuitry in the hippocampus, synaptic input and output paths are diagrammed. (Berridge et al., 2012, modified).

The hippocampus is surrounded by the entorhinal, parahippocampal, and perirhinal cortices. The hippocampus is thought to integrate the information from all the different cortices. It is anatomically positioned to receive and send highly processed information from multiple brain regions. The hippocampal formation itself consists of the dentate gyrus, hippocampus, subiculum, presubiculum, parasubiculum, and entorhinal cortex. It is also part of the limbic system, along with the cingulate cortex, olfactory cortex, and amygdala. Most of the hippocampus's neocortical inputs come from the perirhinal and parahippocampal cortices through the entorhinal cortex, and most of its neocortical output is through the subiculum, which also projects to entorhinal cortex. The hippocampus is also connected to subcortical and cortical structures, including the anterior thalamic nuclei (ATN), the mammillary bodies, and septal nuclei of basal forebrain.

Ramon y Cajal's drawing of a transverse section of the hippocampus in Figure 1B, illustrates the internal anatomical organization. This drawing emphasizes the densely packed pyramidal and granule cell layers as well as the intrinsic details of the pyramidal neurons that give rise to the plastic synapses that are characteristic of the hippocampus. Figure 1A illustrates the tri-synaptic circuit as well as input and output pathways of the hippocampus. The perforant-path carries polymodal and sensory input from layer II of the entorhinal cortex (EC) and synapses onto the dendrites of the granule cells in the dentate gyrus (Neves et al., 2008). The projection of these granule cells onto the proximal apical dendrites of CA3 pyramidal cells form the mossy fibres diagrammed in Figure 1. The Schaffer collaterals are the projections of CA3 to CA1 pyramidal cells completing the tri-synaptic circuit. In addition to this circuit, there is an associative

network interconnecting CA3 cells. Flow of information as shown here is uni-directional, however, there are also direct connections from the EC to CA1 and CA3. Layer III cells of the entorhinal cortex send input signals to distal apical dendrites of CA1 pyramidal neurons, and layer II neurons synapse onto CA3 cells.

The deep layers of the entorhinal cortex are the target of hippocampal output. Hippocampal output projections also go through lateral septal area, connected via the fornix. Sources of hippocampal output include cortical areas such as the prefrontal cortex. The superficial layers of the entorhinal cortex are the greatest source of hippocampal input. Input projections from the medial septal area includes cholinergic and GABAergic signaling, controlling the physiological state of the hippocampus and oscillatory rhythms, ie theta. These projections synapse onto inhibitory neurons, or interneurons sparsely distributed throughout the hippocampus of which basket cells are one type. Modulatory inputs from dopamine, serotonin, and norepinephrine systems travel through the fornix into the hippocampus. As we investigate what gives rise to the synchronous activity generated in the hippocampus it is important to keep in mind connections that expand beyond the tri-synaptic circuit even if they are not the main focus of this thesis.

Discoveries Made From the Hippocampally Impaired and Lesion Studies

A key way to understand the significance of a particular brain region is by removing it or damaging it. Although accidentally, that's exactly how the significance of the hippocampus and medial temporal lobe region was first discovered. Henry Gustav Molaison, referred to as HM, was a patient who suffered from epileptic seizures and had his medial temporal lobe removed (Scoville and Milner, 1957). Post-surgery, patient

HM had reduced seizures, but he also developed anterograde and partial retrograde amnesia. Patient HM was left unable to form any new episodic memories and incapable of learning new facts or recent events. However, his short-term memory and childhood memories remained intact until he died in 2008. Other patients with impaired hippocampal formation have since been studied, and continue to verify the importance of the hippocampus in the formation of episodic memories and ability to remember events (Gabrieli et al., 1997; Henke et al., 1997; Maguire, 2001)).

Numerous sought to and categorize the forms of memory that are affected by hippocampal damage. Some studies state that hippocampal amnesia results in the impairment of long-term memory and preservation of short-term memory (Baddeley, 1970; Cave and Squire, 1992; Shallice, 1988). Others have also demonstrated that learning of associations between items as well as the order or sequence of events require an intact functioning hippocampus (Mayes et al., 2007; Mayes et al., 2001; Spiers et al., 2001). For example, in a task which involved watching a grid in which three objects were serially presented in different locations, patients were able to remember the objects and the locations separately but could not form an association between the objects and the location (Olson et al., 2006). Patients with hippocampal damage have a normal vocabulary and are able to recall facts just fine. This kind of memory is termed semantic memory. Semantic memories then are thought to become independent of the hippocampus and are stored in other brain regions over time (Nadel and Moscovitch, 1997).

A number of theories have been proposed to explain the different memory processes that are affected when the human hippocampus is damaged. The Declarative

Theory states that the hippocampus is only crucial during a time limited period and all memories are ultimately consolidated in neocortical sites (Squire, 1986). According to this theory, facts or events that were learnt long ago, have been consolidated and are spared by hippocampal damage. The Multiple-Trace Theory defines episodic memories as those that are depended on the hippocampus throughout a lifetime and become more resistant to damage through repetition (Moscovitch et al., 2005; Nadel and Moscovitch, 1997). According to this the Multiple-Trace Theory, older memories are represented by more traces throughout the hippocampus and adjacent regions, perirhinal and parahippocampal, and therefore less susceptible to disruption during hippocampal damage. These older memories are thought to 'semanticize', meaning they becomes integrated with pre-existing knowledge (Moscovitch et al., 2005).

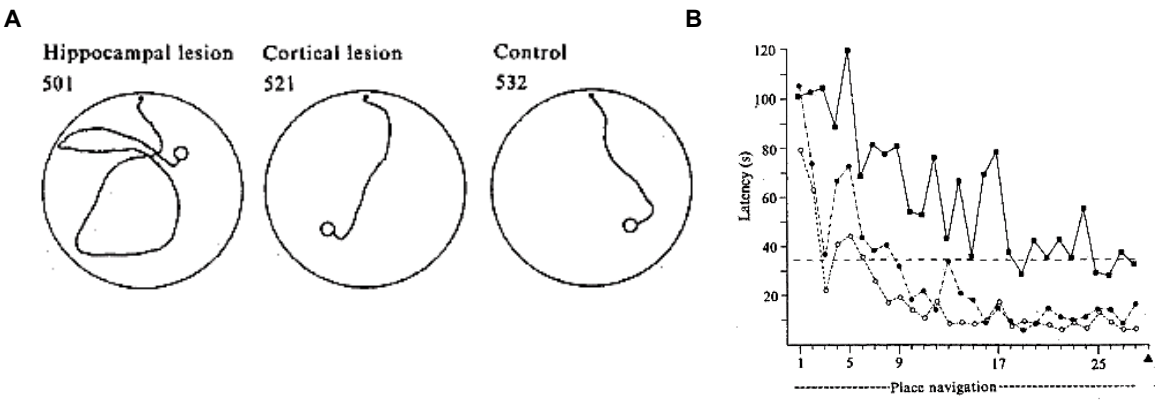


Figure 2. Hippocampal Lesions Impair Place Navigation in Morris Water-Maze. A, The median path of rats with hippocampal and cortical lesions compared to the control group on trial 28. The hippocampal-lesion animals took longer and more circuitous routes to find the hidden platform. B, Mean latency of escape for 50 trials of the experiment in which animals are to find a hidden platform in a pool of water. Diagrammed from top to bottom, hippocampal lesion, cortical lesion, and control animals. With enough training animals with hippocampal lesions eventually learn to escape and find the hidden platform. (Morris et al., 1982).

Lesions of the rodent hippocampal formation have also been found to cause severe effects in learning and memory in a range of behavioral tasks, most of which are spatial and include exploration of mazes such as the radial-arm maze, water-maze, and T-maze (McGregor et al., 2004; Morris et al., 1982; Olton et al., 1979; Rawlins and Olton, 1982; Sutherland et al., 1983). In an open watermaze, rodents must locate a hidden escape platform in a large circular tank. Animals are trained to remember the fixed location of the platform over days, and latency and path lengths are recorded. Figure 3A reveals that hippocampal damage results in the inability of rodents being able to find the hidden platform in a water-maze. Spatial memory is also tested by removing the platform from the pool and measuring in what quadrant the animal spends the most amount of time. In the water-maze, rodents with lesioned hippocampi require extensive training to navigate to the correct quadrant (Morris et al., 1990). This extensive training allows for extra-hippocampal brain structures to eventually acquire the spatial memory. As can be seen in Figure 2B, with time, as the number of trials increase the path to the hidden platform is eventually learned. However, when the position of the hidden platform does not remain constant and changes from trial to trial, overtraining does not help the lesioned animals learn the task as the animal cannot rely on egocentric strategies (Ramos, 2002; Ramos and Vaquero, 2000). These studies suggested that the hippocampus is important for rapid encoding of information.

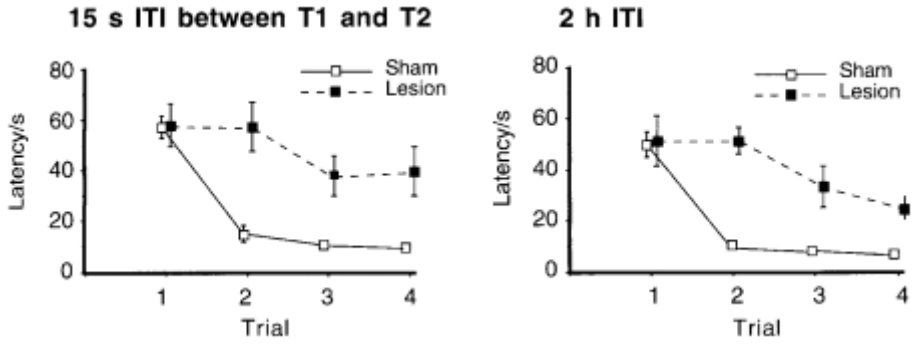


Figure 3. Rapid, One-Trial Learning Cannot Take Place Without a Hippocampus. The delayed acquisition of matching-to-place task (DMP) requires the learning of a novel platform location each testing day. Animals lesioned after 8 days of pre-training failed to show any improvement in escape latency between trials 1 and 2, even when the inter-trial interval was just 15s. In these experiments, ibotenic acid lesions were used, but the same is true when D-AP5, the NMDAR antagonist, is infused into the hippocampus. (Steele and Morris, 1999).

To test rapid encoding of spatial information a common behavioral task used is the delayed matching-to-place version of the water-maze (DMP) which requires the learning of a novel platform location on each testing day. In animals with hippocampal lesions their learning of the DMP task is severely impaired (de Hoz et al., 2005; Steele and Morris, 1999; Whishaw, 1987). Steele and Morris (Figure 3) found that rats that had their hippocampus lesioned after 8 days of pre-training, still did not improve their escape latency between trials 1 and 2 even after 8 more days of post-operative training, no matter the length of the interval, 15s or 2h, between trials.

Lesion studies can either be large, encompassing the whole hippocampus, or more selective. Kainic acid lesions to CA3 result in a deteriorated performance in the 8-arm radial maze (Handelmann and Olton, 1981), as well as DMP performance (Whishaw, 1987). Lesions to the DG also have similar results. DG was found experimentally to be a region of neurogenesis, making it an interesting region of study to understand how

neurogenesis may relate to learning and memory. CA3 has received quite of bit of interest mainly due to its recurrent connectivity and is thought to be specialized for rapid encoding of new information as well as pattern completion or maintaining stable representations in the absence of new inputs (Mcnaughton and Morris, 1987; Treves and Rolls, 1992). CA1 is termed the novelty detector by some as it receives direct cortical information from the EC and input from CA3, sending stored predictions of the current environment (Eichenbaum et al., 1990; Hasselmo and Wyble, 1997). When isolating cortical input from CA3 input into CA1 by cutting the connections between the two subregions and sparing the subcortical connections, animals still demonstrated place memory (Brun et al., 2002). These initial results allowed scientists to conclude that cortical input to the CA1 was sufficient for place recognition, and input from the CA3 mediates rapid learning and spatial recall.

Lesions along the longitudinal axis of the hippocampus have also revealed that dorsal and ventral portions of the hippocampus may encode different information about the same memory. Dorsal lesions have a greater effect on spatial learning than lesions of the ventral region (Moser et al., 1993; Potvin et al., 2006). Ventral lesions impaired fear-related behavior in an elevated plus-maze (Steffenach et al., 2005). The ventral hippocampus is connected with a number of brain regions implicated in motivation, emotion, and executive functions. These connections suggest that the ventral hippocampus is more involved in innate information processing, such as fear-related behavior and anxiety (Kjelstrup et al., 2002) or the utilization of internal cues (Hock and Bunsey, 1998).

Spatial Memory, and Learning

The lesion studies provided much of the evidence for the role of the hippocampus in spatial navigation and spatial memory in both rodents and humans. The behavioral deficits observed in hippocampally lesioned animals along with the discovery of place cells, led O'Keefe and Nadel to conclude that the hippocampus mediates a neural representation of space. They proposed the cognitive map theory, that there were two distinct systems that guided spatial learning and memory using egocentric, self-centered, and allocentric, other-centered, information. That which is egocentric involves using vestibular or proprioceptive cues. Allocentric information requires encoding salient features of an environment independent of the animal's current location. The lesion studies demonstrated that the hippocampus impairs allocentric information but not egocentric spatial memory.

Figure 4. Place Cell in the Hippocampus. Drawing of representative place cell. The black lines show paths the animal took in an open arena. Red indicated the where firing from the place cell took place. (Bird et al., 2008).

The discovery of places cells in the hippocampus, along with grid cells in the entorhinal cortex, head direction cells in the subiculum, and border cells in both allowed scientists to postulate how mental images are constructed (Fyhn et al., 2004; Lever et al., 2009; Taube et al., 1990). Three scientists proposed the Byrne, Becker and Burgess (BBB) model, a computational model of the neural mechanisms that underlie spatial

memory and imagery (Byrne et al., 2007). The BBB model proposes that during the recall of a mental image, place cells reactivate representations of the spatial geometry of the environment and the locations of objects in it. The parahippocampal cortex represents geometric information, distances to environmental boundaries, and the perirhinal represents objects and features. The BBB model predicts the hippocampus is necessary for the construction of spatially coherent image of a remembered scene and with the help of parahippocampal and perirhinal regions allow one to mentally travel though events and spatial environments. Functional imaging studies performed in humans that have been asked to imagine a particular new scene indicate that the hippocampus is both necessary to create imagery of novel environments and to recreate images of experienced environments (Hassabis et al., 2007).

Although there exists extensive research on the molecular mechanisms and there is a general consensus on what the key molecular players are important for learning and memory, there remains much that is unknown about what the neural mechanisms are and how neural ensembles and firing patterns organize during spatial learning and memory. Specifically, mechanistic insights into the different phases of spatial memory, encoding, consolidation, and retrieval remain elusive. In the chapters that follow, these different phases will be examined. The spiking patterns in the hippocampus are monitored as animals perform a natural explorative behavior, undergo pharmacological manipulations, and high-density tetrode recordings are applied.

The NMDA Receptor, LTP, Antagonists and D-CPPene

Excitatory amino acids such as L-glutamate play an important role in brain function through neurotransmission. Neurons form networks through which nerve impulses and signals travel. NMDA (N-methyl-D-aspartate) receptors are one subtype of receptors that L-glutamate binds and are the most studied due to the early discovery of the NMDAR selective antagonists AP5 ((2*R*)-amino-5-phosphonovaleric acid; (2*R*)-amino-5-phosphonopentanoate) and AP7 which were shown to block the induction of long-term potentiation in-vitro at synapses from area CA3 of the hippocampus to CA1 (Collingridge et al., 1983). NMDA receptors are unique in that they are both ligand-gated and voltage gated, and require co-activation by both glutamate and glycine or D-serine (Kleckner and Dingledine, 1988). In addition, the NMDA receptor has a channel blocking particle, $Mg^{2+.}$ Glutamate must bind to postsynaptic NMDA receptor and the membrane potential must be sufficiently depolarized to expel the magnesium block, to allow influx of Ca^{2+} and Na^{+} into the postsynaptic cell to initiate the enhancement of synaptic transmission through downstream signaling and activation of kinases.

A hippocampal slice from a rabbit stained with the original golgi method(Golgi et al., 2001) demonstrated the organization of the hippocampus and the synaptic plasticity was revealed experimentally by Tim Bliss and Terje Lømo in 1973 when they first described long term potentiation, LTP, a change in synaptic responsiveness or strength. This discovery was the first to support the Hebbian theory, which states that learning and memory storage takes place when connections between coactive cells change. LTP-like synaptic changes were found in the hippocampus following learning (Rumpel et al., 2005; Whitlock et al., 2006).

The ability to remember places and associate them with particular events and outcomes, such as reward, is a natural and instinctive behavior that is critical for our livelihood and survival. Behavioral and pharmacological experiments support the hypothesis that NMDA receptor activation contributes to a synaptic plasticity mechanism in the hippocampus necessary for hippocampus-based learning. Manipulations that interfere with NMDA receptor function, such as NMDAR antagonists, electrophysiological saturation of LTP, and CA1-specific deletion of NMDAR subunits, prevent LTP induction, impair spatial learning, and prevent formation of stable place fields (Moser et al., 1998; Nakazawa et al., 2004; Tsien et al., 1996). Intra-ventricular infusion of AP5, an NMDAR antagonist, results in defects in spatial learning in the Morris Water Maze (MWM) task (Morris, 1989). Rats that receive AP5 injections take longer in finding the hidden platform. However, if an animal is pre-trained, AP5 has no effect in the rat's ability to perform the spatial learning task (Bannerman et al., 1995). The caveat of this task is identifying when learning has taken place and distinguishing motor response learning from spatial learning (Feldman et al., 2010). During the one-trial spatial matching-to-place, DMP, task where the platform location changes everyday, animals remain impaired during AP-5 infusion even after pre-training, much like in Figure 3 during lesioning of the hippocampus. These studies suggest that synaptic plasticity is necessary for the rapid acquisition or encoding of information.

In order for AP-5 to reach the brain it had to be infused via mini-pumps directly into the brain due to its poor penetration through the blood brain barrier. The inability to administer the antagonist intra-peritoneally led to the development of pharmacologically improved analogues that could cross the blood brain barrier. CPP ,3-(2-

Carboxypiperazin-4-yl)propyl-1-phosphonic acid, was synthesized as a rigid analog of AP-7 and was determined to be potent, selective, and competitive antagonist of NMDA-type receptors and most importantly could cross the blood brain barrier and be administered intra-peritoneally (Lehmann et al., 1987). D-CPPene, D-4-[(2*E*)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid, is an analog of CPP, and a simple reduction of the double bond would result in D-CPP, as seen in Figure 5. D-CPPene is the most potent, enantiomerically pure, competitive NMDA antagonist (Lowe et al., 1990).

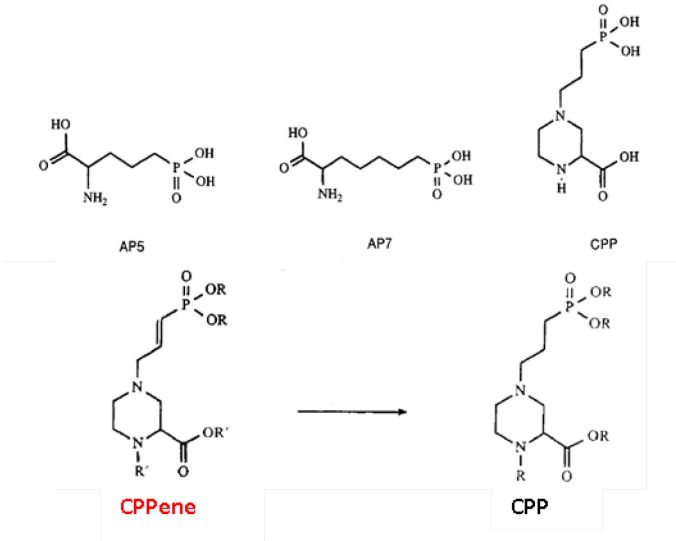


Figure 5. NMDAR Antagonist D-CPPene Derives from D-AP5. Shown is the different chemical structures of the NMDAR antagonists and how they derive from the same D-AP5 structure. The difference in structure is what leads to the difference in specificity of each antagonist. (Aebischer et al., 1989)

The standard form of CPP used in previous studies has been a racemic mixture including both enantiomers (D- and L-) (Ekstrom et al., 2001; Kentros et al., 1998). For both CPP and CPPene the D-enantiomer is 15-20 times more potent than the L-enantiomer. However, upon comparison of D-CPP and D-CPPene spontaneous activity assessed in cortical grey matter slices of Sprague-Dawley rats in Mg^{2+} free buffer, D-

CPPene is 4.7 times more potent than DL-CPP and 2.2 times more potent than D-CPP (Lowe et al., 1990). Potency was determined by assessing threshold concentrations, ED_{50} values, and slopes of concentration-inhibition curves. D-CPPene's blocking ability is completely reversible, just like CPP and AP5. The inhibition exerted by D-CPPene cannot be reversed by perfusion of glycine, indicating that D-CPPene does not exert its inhibitory action at the glycine site.

D-CPPene, unlike other NMDA receptor antagonists, has been used in humans to assess memory retention. Under D-CPPene a significant decrease in number of correctly recalled items in the immediate word recall test 2hrs after administration was found and the effect continued for 8 hrs(Rockstroh et al., 1996). As mentioned, D-CPPene is one of the later developed analogues for NMDA receptor inhibition, and as a result many of the learning and memory studies were first attempted with D-AP5 and DL-CPP. Given the pharmacological characteristics of D-CPPene described, one would expect results to be the same, if not more prominent. One of the few studies using D-CPPene involves measuring the alternation behavior of mice in a Y-maze as a readout of working memory(Parada et al., 1992). In their experimental design, the authors chose to observe behavior 90 minutes after D-CPPene administration. This time point was defined as the time point for maximal efficacy for D-CPPene in experimental models of anxiety, seizures, sedation, and muscle relaxation. Doses of 3 and 5mg/kg of D-CPPene impaired spontaneous alternation. In addition, authors looked at a step-through avoidance task where mice were trained to avoid a dark compartment and retention of passive avoidance was found to significantly decrease when D-CPPene was administered prior to training. This result is very similar to that found in the Bannerman et al., 1995 study, both

indicating that NMDA blocking prior to training results in the inability of animals to learn something novel.

D-CPPene, D-CPP, and D-AP5 in addition to being classified as NMDAR antagonists have also been found to act in different studies as anticonvulsants and muscle relaxants. D-CPPene has been found to have anti-convulsive activity with very long time course after administration (Patel et al., 1990). A reduction in the incidence of sound induced clonic seizures can be observed, with peak anticonvulsant effects observed 1-4hrs after administration and lasting of 1-8hrs when D-CPPene is administered to genetically epilepsy prone rats(Smith and Chapman, 1993). The encouraging results in-vitro and in-vivo using D-CPPene, along with its pharmacological characteristics (high selectivity, enantiomeric purity, reversibility) makes it an appealing therapeutic agent for the clinic. More recently an interest for NMDA antagonists and agonists has increased in the context of neurological and psychiatric disorders. In autism spectrum disorders either reduced or enhanced NDMAR function is implicated, in Alzheimer's disease activation of GluN2B mediates amyloid-B induced alterations in synaptic plasticity, and more (Malinow, 2012; Schmeisser et al., 2012). Whether as a therapeutic agent or a study tool, the use of D-CPPene to mimic NMDAR dysfunction looks to be very promising or at least more applicable than its analogues used in the past.

It is important to mention that although LTP and NMDA receptors have received quite a bit of attention, it is possible that long-term depression (LTD) or decreases in synaptic strength and as well as other mechanisms of plasticity that may or may not be NMDAR dependent are just as important for memory processing (Manahan-Vaughan and Braunewell, 1999; Reisel et al., 2002). For example, the forebrain specific calcineurin

knock-out mice (fCNB1), with reduced LTD and enhanced LTP, can perform the water-maze task but are unable to perform the matching-to-place version of the task (Zeng et al., 2001). In the dentate gyrus and CA3, consolidation of LTP is regulated by noradrenaline, and in the CA1 by dopamine (Swanson-Park et al., 1999). Neuromodulation could also give rise to either a selective strengthening of hippocampal memory traces or to an alteration in the persistence of such traces by its effects on spike time dependent plasticity. In the work included in this thesis, we do no attempt to make any strong conclusions about which synaptic plasticity mechanism is most important and use NMDAR antagonism as a tool to try and experimentally dissociate the different phases of memory processing, encoding and decoding. We are aware that by blocking NMDA receptors systemically, we may be affecting both NMDAR dependent and non-NMDAR dependent mechanisms of plasticity that may or may not occur at the synapse.

Chapter 2: Place Cells

Introduction

Place cells, although first identified in rats, have also been identified in humans and monkeys (Ekstrom et al., 2003; O'Keefe and Dostrovsky, 1971; Ono et al., 1991) and are found in the hippocampus. These cells have a selective place field, a specific location in the environment where they fire, encoding a sense of location. Experience dependent modifications of hippocampal place cell firing have been a subject of study since their discovery. Early work demonstrated that the spatial firing pattern in one environment has no role in predicting the firing pattern in a different environment (Muller and Kubie, 1989). This independent spatial firing of individual cells allows the hippocampus to maintain independent representations of multiple environments(Leonard et al., 1987). These representations become robust with experience. In a novel environment, the ensemble code is initially less robust, but improves rapidly with exploration (Wilson and McNaughton, 1993).

Place cell remapping occurs when the environment changes; place cells become active in different locations, alter their firing frequencies or become silent. However, not all changes in an environment result in remapping. In the case of rotations of selected cues or objects within the testing environment the firing fields rotate with the cues without changing the subset of place cells active(O'Keefe and Conway, 1978). Hippocampal place fields can be dissociated from external sensory landmarks but, as a result of their underlying neuronal circuitry, their relationships remain tightly coupled to each other(Knierim et al., 1998). In a circular environment that changes into a square

through an incrementally changing series of octagons, place fields show fast remapping; displaying an abrupt switch from a circle to a square pattern (Wills et al., 2005). These internal dynamics are thought to arise from the operation of attractors (Tsodyks, 1999; Wills et al., 2005). The attractor hypothesis arose from the knowledge of the auto-associative function of the hippocampal CA3 network. An attractor's defining characteristic is the existence of stable states which are cause by the mutual excitation of neurons within the network and which the system gravitates towards and is described in Figure 6.

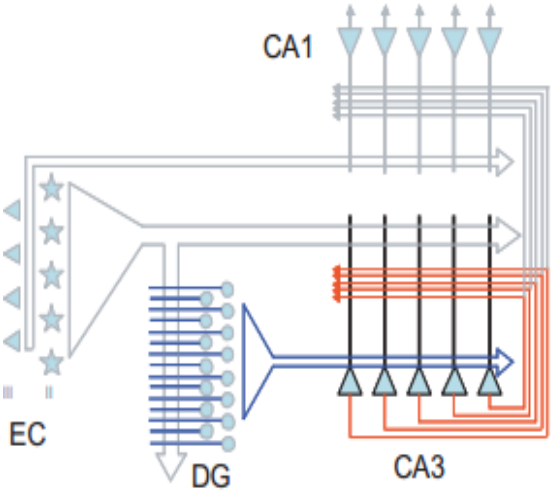


Figure 6. The Attractor Network Theory in the Hippocampus. The EC to CA3 path is thought to drive the place cell representation. Remapping is carried out by the blue connections. Cells in the DG allow pattern separation of the EC input with plasticity in the DG-EC pathway causing remapping. The DG-CA3 imposes new representations onto CA3, and the plasticity in the EC-CA3 pathway allows the EC input to drive the new representation. The red connections mediate the auto-associative network in the CA3. Plasticity in these connections allow a representation to become an attractor. The CA3 representation would then drive the CA1 representation via the CA3-CA1 pathway, and can be compared with the EC-CA1 representation. (Wills et al., 2005)

Previous studies have tried to address the reason behind the remapping of place cells. However, the kinetics and mechanism of remapping is still not known. It is interesting to note that unlike rats, mice have place fields that are spontaneously unstable or constantly remap even after the mouse explores a fixed environment multiple times.

In mice, long-term stability of the spatial firing patterns of place cells depends strongly upon behavioral relevance of available cues such as was observed in the operant place preference task(Kentros et al., 2004). Place cell stability in mice is task dependent and behaviorally modulated.

In a fixed spatial environment, place fields are stable for intervals of up to 153 days(Thompson and Best, 1990). Stable place fields are defined as those that produce firing maps with high spatial correlations during exposures to the same environment. Place cells initially fire in an unfocused or unstable pattern and reach stability with experience. The kinetics and mechanism of the development and maturation of place fields is not well characterized. There is an increase in the size of the mean place field and overall backward shift in the distribution of spikes as the rat continuously runs on a linear track(Mehta et al., 2000). The low firing rate of the place cell at the entrance of the field and high firing rate as it exits the field gives it the asymmetric shape. This asymmetry is reported to reset in the CA1 place cells everyday or when the rat runs in a novel environment. Hippocampal place cells have access to recent memory as was shown in the light- dark experiments. Place cells adopt a place field map or firing pattern in the dark which remains unchanged when lights are turned on, indicating that place-cell activity can reflect a memory of an earlier condition(Quirk et al., 1990).

A place field rate map defines a group of place cells that are active in a particular environment. During exposure to a novel environment, this ensemble of place fields changes. The change in the shape of a testing environment, from a cylinder to a rectangle, is reflected in the spatial firing patterns(Muller and Kubie, 1989). Partial remapping is also seen when the testing environment is scaled up by a factor of two. Does remapping,

then, reflect the magnitude of the difference between environments? If a rat were to run continuously on the same track, we would expect there to be no remapping because the environment would be fixed. A previous study by Leutgeb et al., 2005, addressed remapping by comparing the location and rate of firing of place cells in two conditions: variable cue-constant place, and variable place-constant cue. Spatial similarity was determined by using correlations between pairs of rate maps. Rate similarity was calculated by dividing the relative change in the mean rate by the sum of the mean rates. They found that when the same prominent environmental features were encountered in two distinct places the place cell peak firing locations were uncorrelated and had undergone global remapping. However, when the changes in prominent features were in the same spatial context, or fixed environment, there were mainly rate changes observed, but no changes in the distribution of firing locations(Leutgeb et al., 2005).

Systemic administration of NMDAR antagonist CPP is reported to have no effect on the formation and short term stability for 2-3 hours of CA1 place fields in a novel environment but does have an effect on long term stability, overnight(Kentros et al., 1998). Moreover, in a familiar environment, place cells are as stable after CPP injection as they are after saline injection both short-term, same day, or long-term, overnight. Blocking NMDA receptors in the rat does not seem to prevent remapping in a novel environment, however the CA1-NR1-knockout mice display enlarged and diffused CA1 place fields and show impairments in the Morris water-maze task(Tsien et al., 1996). By analyzing single unit activity, ensemble activity, and population reactivation while blocking NMDAR using a stronger antagonist, we aim to define how the loss of NMDA

receptor function affects the activity and stability of place cells during running and in replay and how it relates to spatial memory processing.

Methods

Behavioral Pre-Training

A total of 4 male Long Evans rats weighing 450 – 550grams, 10-20 weeks old were used in this study. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee and followed US National Institutes of Health animal use guidelines. Animals were first habituated to daily handling and food deprived to 85-90% of their baseline weight and pre-trained to run back and forth on a 1.8m linear track to receive a liquid chocolate-flavored reward (Carnation). The tracks had two reward delivery wells at the ends. Rats were trained on a separate linear track designated for training and in a room different from the recording room. Each training session lasted 30-45min or when rats completed 20-30 laps. Rats only received a reward if they completed a full lap. Following recovery from surgery animals were food-deprived again and retrained on the training linear track with recording cables attached for approximately two days before recording sessions began. Upon completion of all recording sessions, micro-lesions through each electrode tip were made to mark the tetrode locations (30μA for 3sec).

Experimental Design

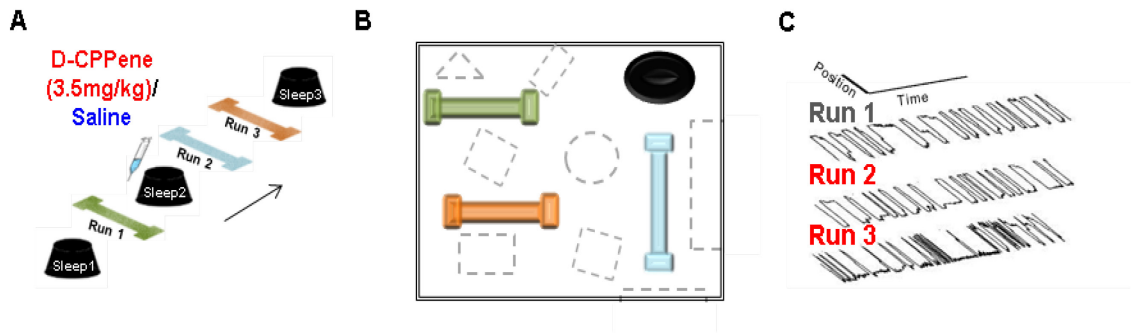


Figure 7. Experimental Design. A, Each experimental day begins with a sleep1 session, and saline or D-CPPene is administered after Run1, during Sleep2, and animals run consecutively on two additional tracks before the final sleep session begins. B, This diagram shows the top view of the recording room arrangement where the tracks are completely novel each experimental day, and the objects in between the tracks are also new and keep the animals from seeing unto the different tracks. C, Diagram of the position tracking and running behavior on the different tracks under D-CPPene.

To investigate the role of NMDAR-dependent plasticity in the processing of spatial memory, we applied high density electrophysiological recording techniques while animals engaged in exploration of novel spatial environments in the presence or absence of a competitive NMDAR antagonist or saline control. A miniaturized microdrive holding 40 independently adjustable tetrodes was implanted in four rats, with tetrodes targeted bilaterally to dorsal hippocampal area CA1. Across multiple recording days, spike data, local field potentials and behavioral position data were collected, and an average of 107 excitatory hippocampal neurons were isolated per session. Each recording day (Figure 7A) began with a rest period ("Sleep 1") followed by exploration of a novel track ("Run 1"), immediately after which animals were injected systemically with either 3.5mg/kg D-CPPene, (D-4-[(2E)-3-Phosphono-2-propenyl]-2-

piperazinecarboxylic acid) or saline and allowed to rest for 40+ minutes ("Sleep 2"). Subsequently, animals explored two additional novel tracks ("Run 2" and "Run 3"; followed by a final rest period ("Sleep 3"). The use of two consecutive post-drug behavioral runs allowed for variability in the kinetics of drug uptake under systemic delivery. Novel environments were created each day by reconfiguring cues in the recording room which remained fixed for the entirety of the recording day (Figure 7B). Throughout the entirety of the experiment, rats would never repeat an experience on the same track. Each recording day the rats experienced three completely novel tracks in different locations of the room surrounded by novel distal cues that the rats had never before experienced. Each track was 1.8m long, 6cm wide, with two 15cm long end areas each 13cm wide where the reward delivery wells were located. The rat's position was tracked using two LED diodes (red and green) mounted on the microdrive and detected by an overhead camera capturing the behavior at 30Hz.

	Run1	Run2	Run3
Average Firing Rate (Hz)	4.819±.70 3.647±.52	3.962±.36 4.115±.36	3.554±.70 3.517±.32
Velocity (cm/s)	13.31±3.71 12.42±2.28	13.02±3.79 19.04±2.39	10.21±2.50 21.53±4.14
% Time Stopped	81.31±3.67 78.78±2.72	84.38±1.92 86.10±3.72	79.00±5.90 87.22±3.15
Number of Laps	28.17±2.93 28.33±2.45	26±2.63 28.5±5.34	27±3.39 32.33±5.21

Saline D-CPPene

Table 1. Rat Behavior. Average firing rates and behavior (mean ±SEM) was quantified by calculating velocity, percentage of time spent stopped on track, and laps per run session. No significant differences were found across or within conditions, pre (Run 1) and post injections (Run 2 and Run 3). *P*-Value (Two-Way ANOVA): Average Firing Rate, $F(2,1186)=0.834$, $P = 0.4347$. *P*-Values (Wilcoxon rank-sum test): Velocity, saline, R1 vs R2, 0.5887, R1 vs R3, 0.5887, R2 vs R3, 0.7831; D-CCPene, R1 vs R2, 0.8182, R1 vs R3, 0.1320, R2 vs R3, 0.0931. Time stopped, saline, R1 vs R2, 0.9372, R1 vs R3, 1, R2 vs R3, 0.4177; D-CPPene, R1 vs R2, 0.1797, R2 vs R3, 0.1797. Number of laps, saline, R1 vs R2, 0.9156. R1 vs R3, 0.8182, R2 vs R3, 0.5173; D-CPPene, R1 vs R2, 0.5693, R1 vs R3, 0.8203, R2 vs R3, 0.7900. (n = 6 sessions across 4 rats).

D-CPPene, the most active enantiomerically pure competitive NMDA antagonist with increased potency and selectivity over other CPP derivatives, is effective for at least 3 hours at the dose used, thus well into Sleep 3 (Kentros et al., 1998; Lowe et al., 1990). Given the kinetics of D-CPPene activity, coupled with the timing of Run 3 (all sessions for Run 3 started on average 85 minutes and ended 125 minutes after injection), we identified Runs 1 and 3 as periods of time with unimpaired NMDA receptor function and maximal NMDA receptor inhibition, respectively. Neither saline nor D-CPPene administration significantly affected general behavior on the track or average firing rates

of the recorded neurons (Figure 7C, Table 1). This is the absolutely critical fact that allows us to interpret our neural activity changes as a function of NMDAR changes and not as a trivial byproduct of behavioral changes.

Surgical Implantation and Tetrode Adjustment

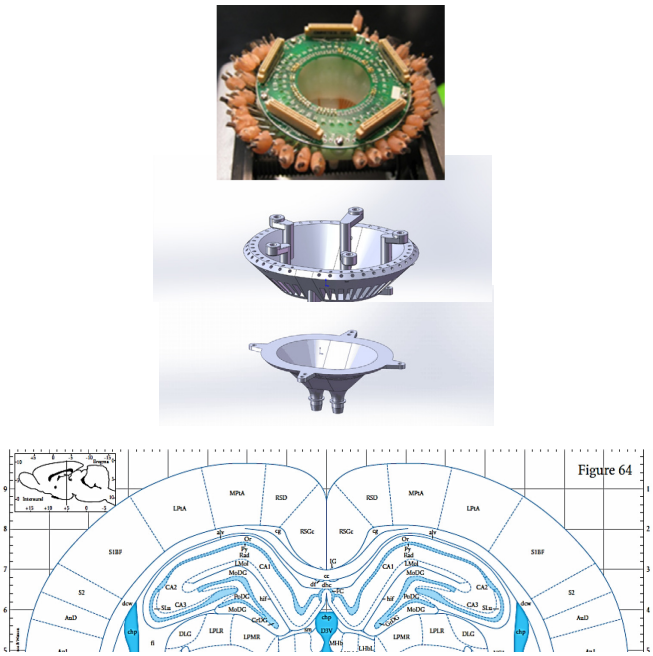


Figure 8. The Microdrive. The microdrive is composed of 40 "top pieces" through which tetrodes flow, surrounding an electronic interface board (EIB) that allows for a total of 160 channels to be recorded simultaneously. The two cannula on the drive are aimed at the dorsal CA1, and sits at the top of the skull. The turning of the top pieces allows for the tetrodes to travel through the brain and into the region of interest.

Rats were trained for 2-3 weeks and surgically implanted with a microdrive array (25-30g) with 40 independently moveable, gold-plated tetrodes, 20 on each hemisphere. Tetrodes were gold plated to an impedance of <150 MOhms prior to surgery. Each tetrode consisted of groups of four twisted 17.8 µm platinum/10% iridium wires from Neuralynx bundled together. Tetrodes were aimed at dorsal hippocampal region CA1 (4.00mm AP and 2.8mm ML) and a bone screw attached to the skull served as the ground. Placement of tetrodes and recordings were performed as previously described (Foster and Wilson, 2006). The tetrodes were slowly lowered into the CA1 pyramidal layer over a 1week period using characteristic EEG patterns (ripples and sharp waves)

and neural firing patterning as guide into the pyramidal cell layer. The advantage of the use of this microdive and tetrodes is that it allows for simultaneous recording of hundreds of single units.

Pharmacology

Rats received either a saline or 3.5mg/kg i.p. injection of D-CPPene (D-4-[(2E)-3-Phosphono-2-propenyl]-2-piperazincecarboxylic acid, Tocris Bioscience). If rats were injected with D-CPPene the next recording would take place 48 hours later to make sure that the drug had completely washed out. D-CPPene is a potent and competitive NMDA receptor antagonist. D-CPPene is an analogue of D-CPP, 2.2 times more potent than D-CPP and 4.7 times more potent than the racemic mixture DL-CPP (Lowe et al., 1990). The calculated potencies indicate that a lower concentration of D-CPPene is required to saturate the NMDA receptors and the dissociation constants reveal that less drug is required to occupy 50% of the receptor and produce the physiologic effect (Lowe et al., 1990). Kentros et al., determined the effect of a 10mg/kg dose of DL-CPP and reports that DL-CPP blocks primed-burst potentiation, increased CA1 spike population amplitude produced by electrical stimulation of hippocampal commissure, at 90min and 180min after systemic administration, and is no longer effective after 24hrs(Kentros et al., 1998). Given the chemical characteristics and reported potencies of each analogue, the 3.5mg/kg dose of D-CPPene administered systemically in this study would equate to a 16.5mg/kg dose of DL-CPP. We can then infer that in our experimental design, D-CPPene was effective for at least 180min, when animals were well into sleep 3.

Data Acquisition

All data was collected using the Digital Lynx data acquisition system (Neuralynx). Analog neural signals were digitized at 32556 Hz. Spike threshold crossings (50 μV) were recorded at 32556 Hz. Continuous local field potential data were digitally filtered between 0.1 and 500 Hz and recorded at 3256 Hz. Cells were isolated manually using the spike waveform clustering program XCLUST (xclust2, Matt A. Wilson). Only well isolated clusters not corresponding to inhibitory neurons were used in the data analysis.

Place Field Analysis

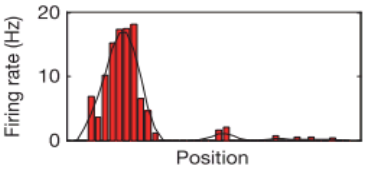


Figure 9. Plotting a Place Field. A place field is plotted as a histogram of spikes along position normalized by occupancy, or time spent at each position.

Position was linearized and binned (2.5 cm). Place fields were calculated as the number of spikes fired in a particular position bin divided by the time spent in that bin, smoothed with a Gaussian kernel with a standard deviation of 5cm, and identified when the peak firing rate of the pyramidal cell along the position bins was greater than 1 Hz. The place field size was defined as the area for at least 10cm(Ekstrom et al., 2001) bounded by the points where the firing rates fell to less than 10% of the peak rate. Sparsity was calculated as a measure of spatial selectivity, to determine how diffuse the place cell firing was along the linear track:

$$\text{Sparsity} \quad = \quad < f >^2 \ / \ < f^2 >$$

where f is the firing rate, and the expectations are calculated across all positions.

Results

Place Fields Only Weakly Affected By NMDAR Antagonism

To determine if NMDAR antagonism affected the hippocampal representation of space, we examined the place-selective firing characteristics of the recorded unit, place cells. Place fields under D-CPPene (Runs2 and 3, red) were larger (Fig. 10C,D) and more diffuse, as measured by sparsity(Jung et al., 1994), compared to place fields during pre-drug exploration (Run 1). Place field characteristics in saline sessions did not change between runs. Even though the place fields were slightly affected the place field map, which is simply the alignment of all place fields by their peak firing, still appears to accurately portray the track.

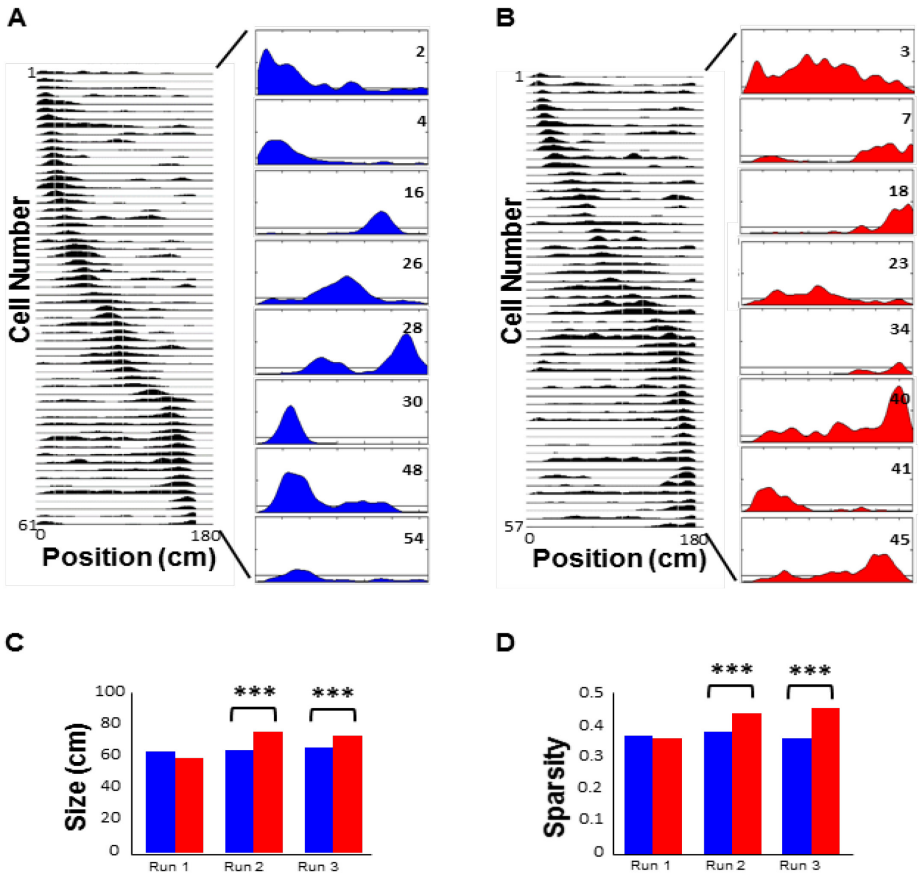


Figure 10. Effect of NMDA Receptor Antagonism on Place Fields. Representative rate maps and place fields during Run2 post administration of saline, A, in blue and D-CPPene, B, in red. Place fields Y-axis is constant (Firing Rate 0-25Hz). Rate maps Y-axis is normalized. Group data place field size, C, and sparsity, D, in saline and D-CPPene during all three Runs (mean ±SEM) (n=6 sessions, 4 rats). * *P*<0.001(Wilcoxon rank-sum test).**

In order to test whether spatial information was available in the place fields under the effects of the NMDA receptor antagonist, we performed memory-less Bayesian position reconstruction to estimate position during running behavior based on the unit place fields and population-level spike patterns(Davidson et al., 2009). A probability-based decoding algorithm was used to estimate the rat's position throughout the experiment based upon the unit place fields and the spike trains. The probability of the

animal's position (*pos*) across *M* total position bins given a time window (τ) containing neural spiking (*spikes*) is

$$\Pr(pos, spikes) = v / \sum_{j=1}^{M} v$$

where

$$v = P(\prod_{i=1}^{N} f_i(pos)^{n_i})\, e^{-\tau \sum_{i=1}^{N} f_i(pos)}$$

and *P* is the (uniform) prior probability over position and $f_i(pos)$ is the position tuning curve of the *i*-th unit, assuming independent rates and Poisson firing statistics for all *N* units. A time window of 250 ms was used to estimate the rat's position on a behavioral timescale. A time window of 20 ms was used to estimate position during candidate population events.

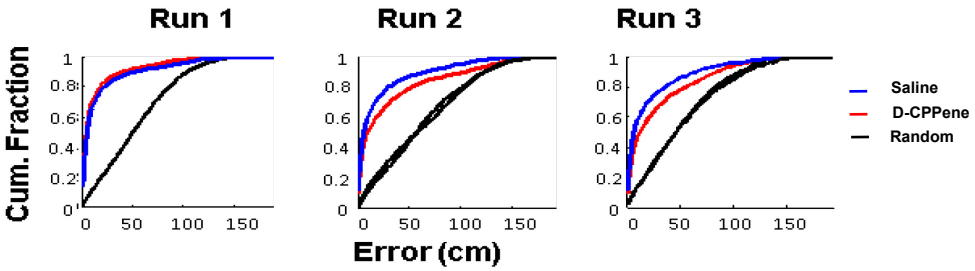


Figure 11. Position Reconstruction During Run. Cumulative distribution functions of the error of the maximum posterior probability estimate of position for saline and D-CPPene group data (all sessions). Black traces represent when place field locations were randomized and correspond to a chance level of decoding. *P*=0, (Kstest2 and Wilcoxon rank-sum) when comparing saline and D-CPPene conditions to randomized locations.

Position estimation was significantly better than chance for all runs, including both Runs 2 and 3 under drug (Figure 11). Thus, the relatively subtle effects on position-specific firing caused by D-CPPene on place fields were not sufficient to prevent spatial information encoding by the hippocampal place cell population. The decoding of run results indicate that place field activity still contains information about location along the track even under NMDAR antagonism.

Place Fields Are Stable and Selective in Novel Environments

To test the place field stability and selectivity between multiple environments we measured spatial correlation of place field firing from the same cells across runs and within runs for both saline and under NMDAR antagonism (Figure 12). The correlation values drop significantly when looking across different tracks, or different runs, meaning as the diagram demonstrates in Figure 12 that across environments a place cell does not fire or form a place field in the same place or location along the different environments, in this case different tracks. Place cells change location when experiencing different tracks, for both the saline and D-CPPene conditions. Each run takes places on a different track in a different part of the experimental room and surrounded by very different objects and cues, and the selectivity of the place field location along the track reflects that environmental difference among the Runs. When the place field formed after the first ten laps is compared to the place field formed after summing up the firing activity of the last ten laps, the correlation values are much higher, meaning the place fields do not change significantly during the time period of running on one track. The same is true when comparing the places fields formed when using the spike activity from the odd laps to that of the even numbered laps. These results indicate that place cells are not only highly

selective and specific but also stable during the experience and running of each individual track. It is also important to note, that under NMDAR antagonism, although the spatial correlation values are significantly lower than those of the saline control animals when looking at place cell firing within individual runs, the values are significantly higher than spatial correlations across runs, indicating that some place field selectivity and stability is preserved under NMDAR antagonism.

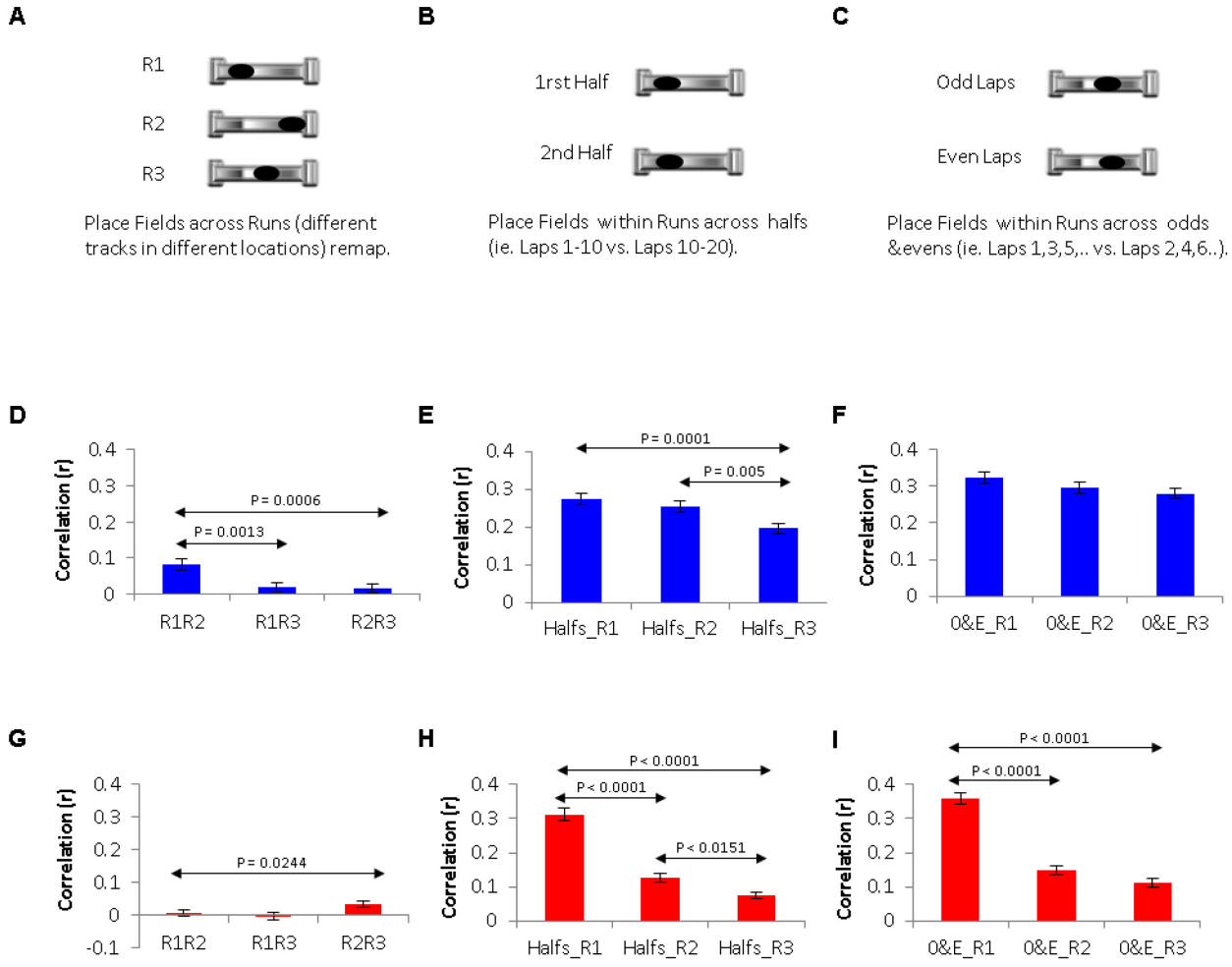


Figure 12. Place Field Stability and Selectivity. Illustrated are diagrams of how a place cell fires in separate environments across different tracks, A, and within the same track across laps, B and C. Spatial correlations (mean ±SEM) of the group data for each situation are plotted below for both conditions, saline (D,E,F) and D-CPPene (G,H,I).

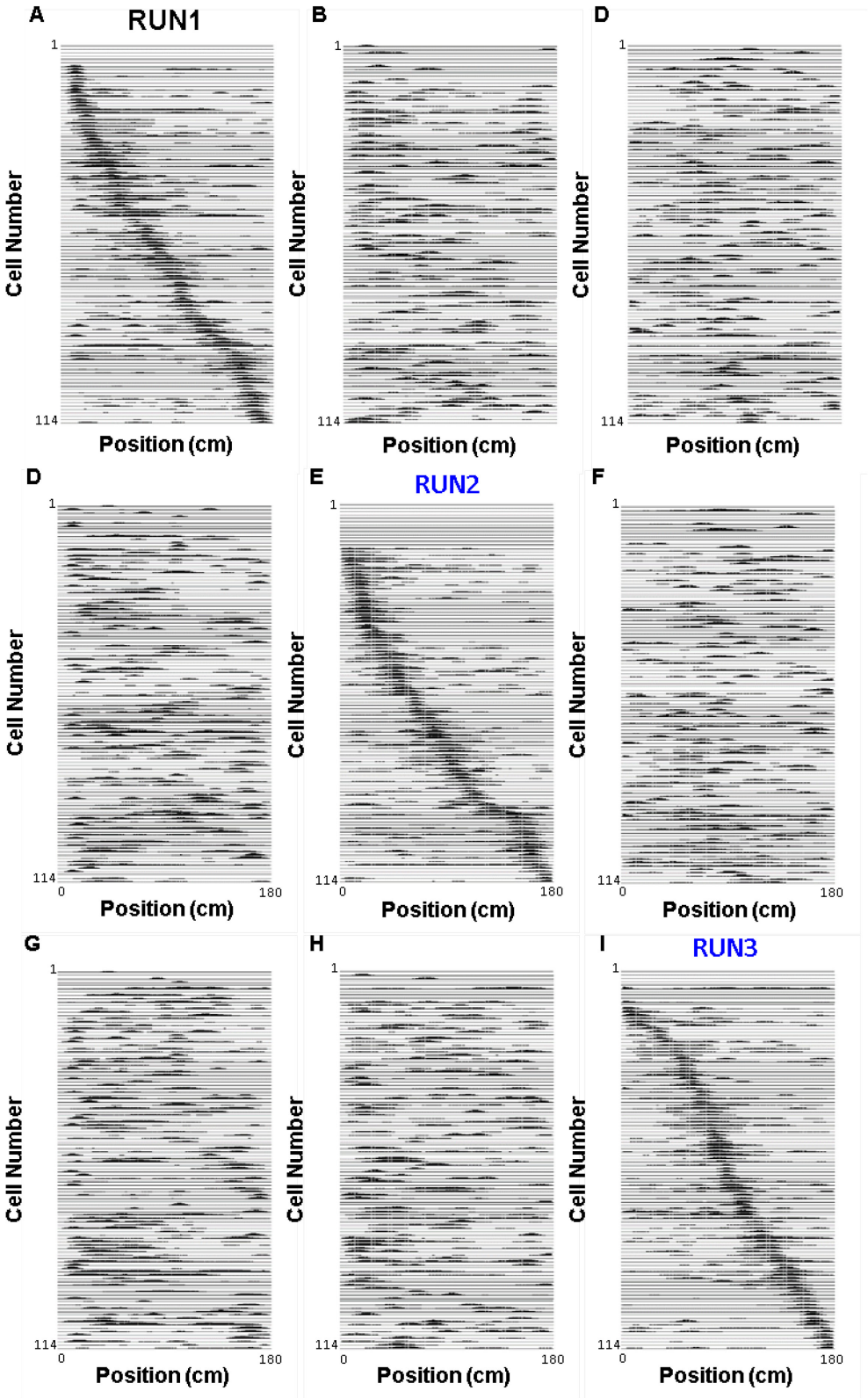
Another way in which we can verify that each of the three tracks recruits a unique population of place cells, is by creating place field rate maps across tracks as is demonstrated in Figure 13. There are 114 place cells that are present throughout the entirety of one experimental recording day, including runs and pre and post sleep sessions. We ordered the place cell ensemble by their peak firing rates for one individual run and use that same unique order of place cells and see where the place cell fires while the animal runs on the other two remaining tracks. Just as the spatial correlation values predicted, we observed that when place fields are arranged by their peak firing in Run1 they change location during Run2 and Run3. Place cells remap; some become silent, others increase their firing, and some change their location of preferred firing. Figure 14 shows the rate maps under NMDAR antagonism and further validate that place cell selectivity and specificity is preserved. We have also plotted rate maps within sessions, Appendix A and Appendix B. Although the rate maps or the place fields are not identical they are very similar and the entirety of the track is represented in both maps. It is interesting to ask why maps are not identical. It is also important to note that these tracks are novel, meaning that although they are all the same width and size, the animals have never experienced them before and each one is in a different part of the room and identified by a completely different set of distal cues.

The low spatial correlation values across tracks as well as the rate maps indicate that there is little overlap in the spatial representation of the tracks. To test whether the spatial information content of the firing is selective and as specific as the location of place fields, we performed cross-track run decoding. In other words we tried to decode the running of the current track using the spikes and place cell firing activity of a prior

track. We observed an increase in error and an inability to decode position accurately (Appendix C).

The length of time it takes for environment to become familiar has not been strictly defined experimentally. The slight differences in place cell firing activity when comparing laps within the same environment could be due to the existence of non-spatial firing patterns. The activity of these pyramidal cells in the hippocampus termed place cells can be influenced by more than location (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971). After all, the hippocampus has different cortical inputs and any one cell can have a diverse combination of inputs. If a learned environment can be remapped, this would indicate that a familiar environment can be encoded by place cells in multiple ways, generating multiple place field maps for the same environment that would encode more than spatial information. This then begs the question of how are these place field maps committed to memory. To test this we look at reactivation patterns and replay in the next chapter.

Figure 13. Cross-Rate Maps Under Saline. The same 114 place cells are arranged by their peak firing rate for Run1 for all the different tracks cross the first row. Arranged by their peak firing for Run2 (D-F), and by Run3 (G-I). The firing rates are normalized so that the y-axis is the same between compared runs.



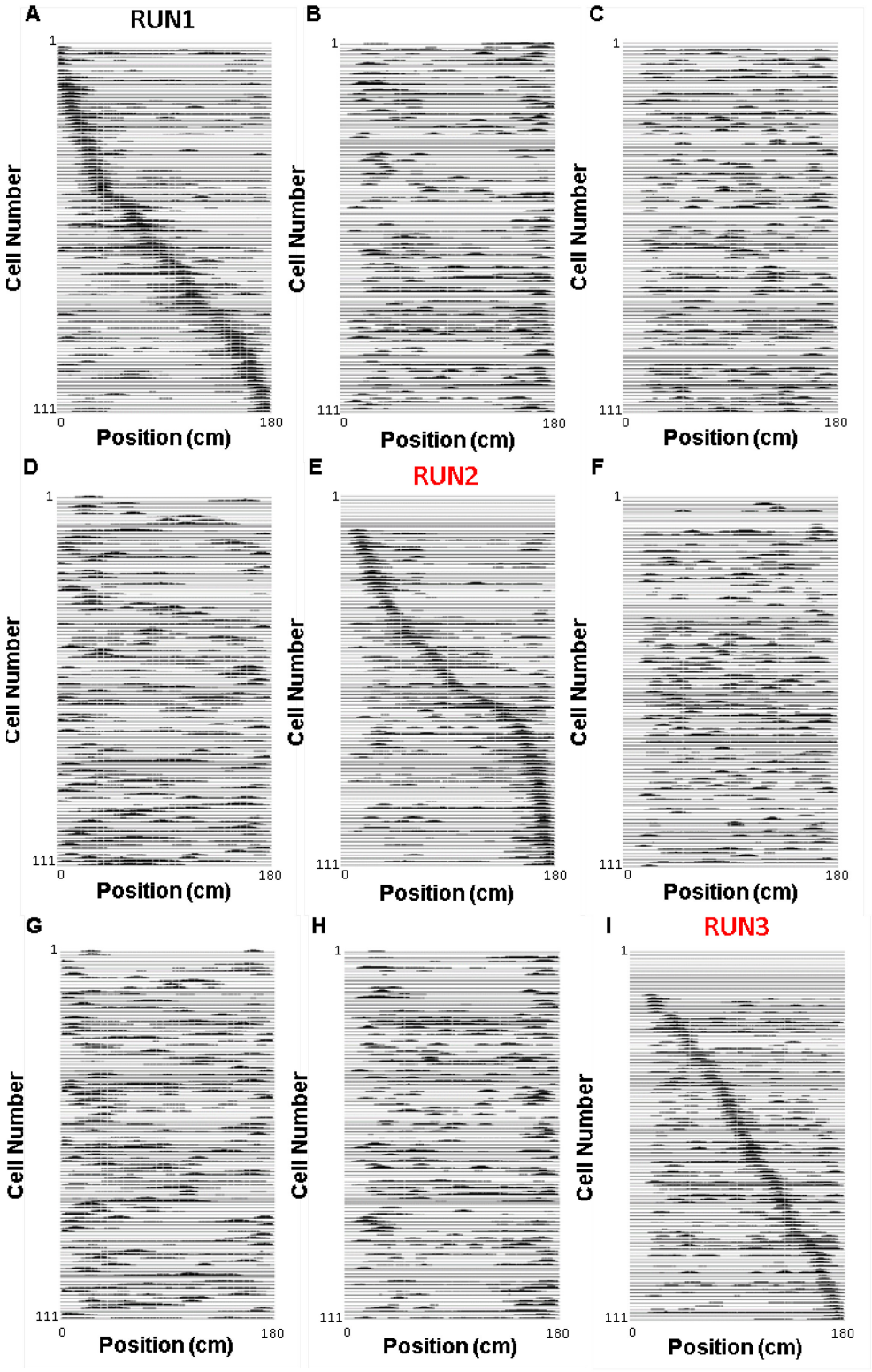


Figure 14. Cross-Rate Maps Under D-CPPene. The same 111 place cells are arranged by their peak firing rate for Run1 for all the different tracks across the first row. Arranged by their peak firing for Run2 (D-F), and by Run3 (G-I). The firing rates are normalized so that the y-axis is the same between compared runs.

Chapter 3: Replay Encodes Spatial Memory

Introduction: Linking the Place Fields

Place fields were the first demonstration that information could be encoded in the firing of pyramidal cells in the hippocampus. However, it is not easy to dissociate the encoding and retrieval phases of episodic memory using place field firing alone. Replay sequences offer exactly this. Our animals perform a behavioral task capable of testing the acquisition as well as the retrieval of spatial memory with a short enough time frame to distinguish between place fields and sequences. Our recording technique also allows us to study large numbers of cells simultaneously to be able examine the fast encoding of replay of experiences even after single trials. In this manner, we can compare several measurements of place cell activity including: place fields, run-time decoding, and replay. All are place cell phenomena, not place field phenomena. Place fields and replay rely on completely different spiking events, allowing us to ask the question if the two diverge in plasticity mechanisms. We set forth the hypothesis that if place-cell replay sequences are a critical mechanism of hippocampus dependent memory, then replay should fail to occur for behavioral sequences experienced under NMDAR blockade. We provide the data that allows us to propose, by analogy with hippocampus dependent behavioral memory, that replay might become independent of NMDARs once memory has already been established.

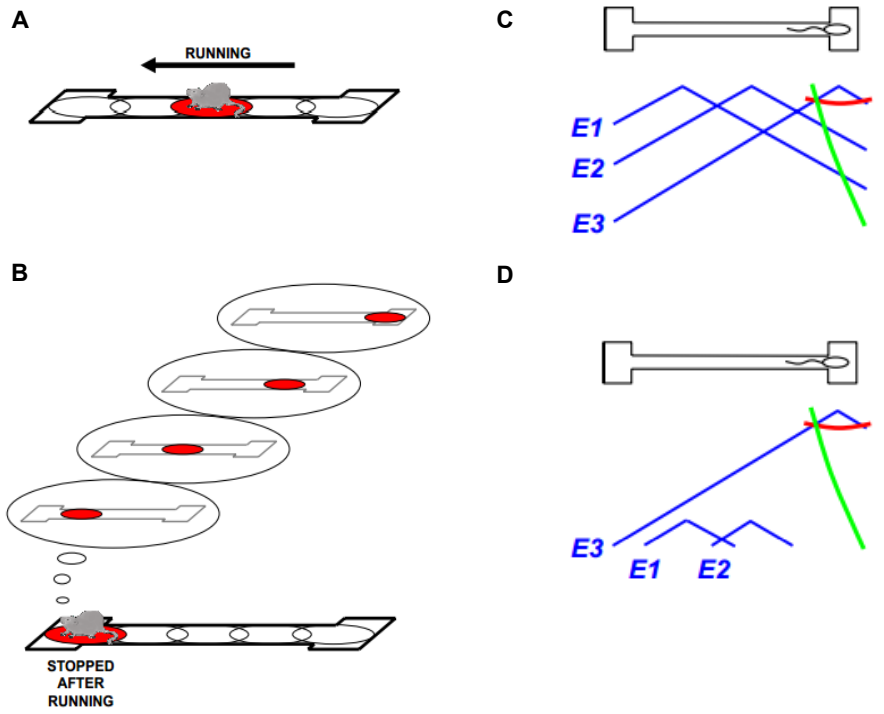


Figure 15. Model of Replay. A, While a rat runs along a linear track, hippocampal neurons are active in a place-specific manner. In this example, the preferred places of five different hippocampal place cells are shown as ellipses on the track. B, As the rat runs through successive locations on the track, place cells associated with these locations become active, as indicated by the red shading. When a rat stops at the end of a linear track after running down it, the sequence of cell activity experienced during running is replayed in reverse order. C, When an animal reaches the end of the track, after having traversed the track causing the excitatory cells, E1, E2, E3 to fire. The firing of these cells both reflect a spatial tuning derived from their inputs as well as an activity dependent bias which may be provided via synaptic potentiation or intrinsic excitability increases. Activity at the end of the track is controlled by inhibition mechanism. During the non-sharp-wave-state inhibition is high (red line), when sharp waves occur, a drop in inhibition takes place (green line) and reverse replay takes places as the sub-threshold fields of the cells that were once active on the track are now revealed. D, It is imperative for the animal to run through the track in order to have replay. In the absence of experience, or prior excitatory input, the drop in inhibition fails to reveal any activity. (Foster et al., 2006)

Foster et al.2006, reported the first observation of sequential awake replay. While animals experience a linear track place cells fire according to their preferred firing location along the track. When the animal reaches the end of the track and stops as it consumes a reward, sequences of place cells originating at the animal's current location are reactivated in the reverse of the order experienced behaviorally. These sequences are temporally condensed occurring in just 100s of milliseconds. The discovery of replay adds further evidence to the theory that hippocampus-dependent memories are likely to take place in two main stages(Buzsaki, 1989). First, the hippocampus rapidly encodes memories in the awake state. During offline periods memory traces are reactivated to facilitate the transfer to longer term storage in cortical networks or to strengthen associations between traces in different regions. Replay can be seen after the first time an animal runs along a track, suggesting that replay may important for one-trial learning and continues to exist post-experience.

Reverse replay may reflect a pattern of increased synaptic strengths distributed across groups of neurons necessary for spatial memory formation. By disrupting plasticity, we hope to disrupt replay and memory trace establishment, thereby unraveling the mechanisms behind the encoding and retrieval of spatial memories. Figure 15C and D, demonstrate a possible mechanism. Diagrammed are three different places cells that receive different excitatory input and different combinations of synaptic weights during the running which provide both their spatial tuning and activity dependent bias. When an animal reaches the end of the track, cell activity is controlled by inhibition. In sharp-wave mode, the green line, inhibition is low allowing the sub-threshold fields of the cells to fire in reverse order. Figure 15D highlights the importance of enhanced excitatory

input, carried out through synaptic potentiation or intrinsic excitability increases, for replay to occur. Wilson and McNaughton, 1993, revealed inhibitory interneurons are suppressed during the development of novel spatial representations which may in turn facilitate the synaptic modification necessary to encode new spatial information in the form of replay(Wilson and McNaughton, 1993).

In order to observe replay, large populations of neurons must be recorded simultaneously. As mentioned, our tetrode recordings together yield 100s of simultaneously recorded cells. When only a small population of neurons is recorded, pairwise measures are used to identify synchronous reactivation of place cells that were active during running(Cheng and Frank, 2008; Jackson et al., 2006; O'Neill et al., 2006). However, the sequential structure is not captured nor is the entirety of the trajectory along the track when only looking at pairwise measures. Pairwise studies reveal that pairs of place cells that are active during the running on a track, are reactivated during post sleep/rest. By looking at replay events we are looking at more than pairs of cells that are reactivated and can begin to characterize the firing pattern and structure of an ensemble of place cells that are synchronously active. The field generally interchanges the use of the terms reactivation and replay. In our work, replay only reflects reactivation of sequences of place cell activity. We go one step further and define different types of replays and introduce trajectory events.

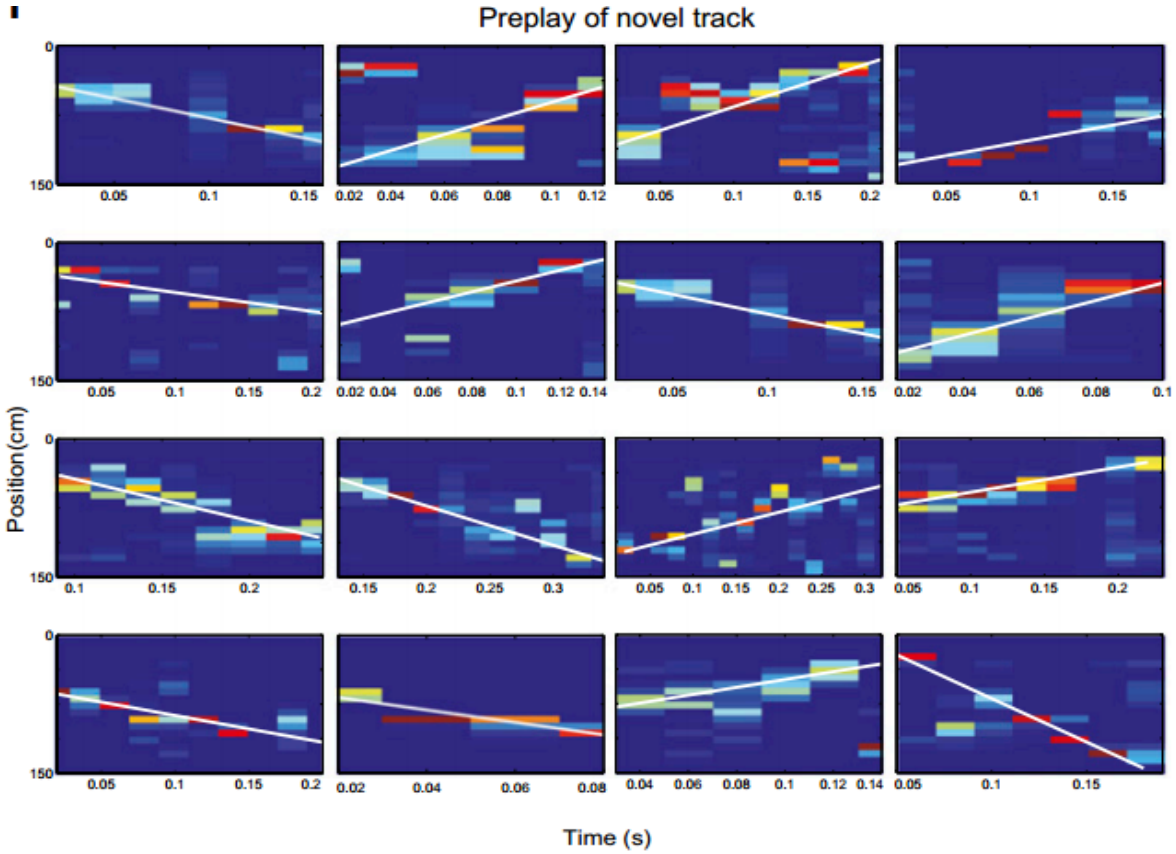


Figure 16. Preplay. Examples of significant decoding of a future trajectory on the novel track from ensemble place cell activity of 31 cells during the sleep/rest session before novel track exploration (i.e., prerun sleep/rest) in one naïve rat (20-ms bins, animal velocity <1 cm/s). The white lines show the linear fit maximizing the likelihood along the virtual trajectory. (Dragoi and Tonegawa, 2013)

An interesting phenomena that has recently received quite a bit of attention, is the idea that the expression of sequence activity may be independent of experience and may pre-exist. Dragoi and Tonegawa, found in both rats and mice that CA1 firing sequences during periods of awake rest are correlated with sensory-induced sequences in an environment that is experienced only later, and termed this preplay (Figure 16) (Dragoi and Tonegawa, 2011, 2013). They propose that preplay may facilitate future learning when a new experience is introduced with multiple steps of increasing novelty. The model of preplay suggests sequences pre-exist in the hippocampal network and are recruited for encoding new memories. If sequential activity is not driven by specific

sensory experience and does not correspond to previously experienced sequences then it is possible to imagine that animals can mentally explore all trajectories that were replay without ever experiencing them. Figure 17, summarizes what is known about offline sequence activity from a number of studies in familiar and novel environments. The greatest amount of offline activity is observed when a novel environment is experienced for the first time and continues to increase with experience, but as the environment becomes familiar the amount of offline activity decreases. In sleep, the general consensus is that offline activity decays with time. The change in r represents preplay that may exist prior to experience. Preplay may just have gone unnoticed prior to Dragoi's study due to the assumption that any correlation between run and pre-sleep was not real and was subtracted from the correlation between run and post-sleep(Tatsuno et al., 2006).

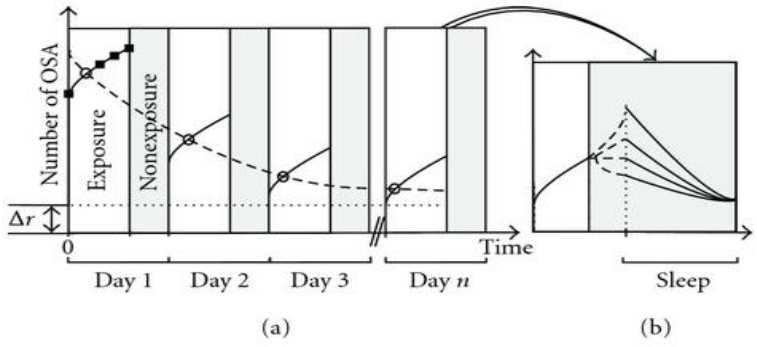


Figure 17. Reactivation as a Function of Experience. (a) Offline activity, or reactivation and replay, increases with the length of exposure within a day (solid line segments). Across days as the environment becomes familiar, the level of offline activity decreases (dashed line). The Δr represents the amount of intrinsic activity or preplay. The grey-shaded columns represent activity post-experience and are summed up in (b). The dashed lines at the beginning of sleep, representing the transition from exposure to the beginning of sleep, which as the image portrays, is an area in which there are multiple theories and studies need be performed. However, the general consensus in the field thus far, is that offline activity such as replay decays over time in sleep. (Buhry et al., 2011)

Although preplay has generated enormous interest, it is important to note that these findings have only been reported by one laboratory. In our study, we investigated preplay in the context of NMDAR dependent plasticity, and compared the structure of preplay to that of replay, and find that replay is better described as trajectory events that encode a continuous moving trajectory. Because we record from a larger population of cells simultaneously we are able to measure the occurrence of preplay sequences much more readily.

Our recording technique also allows us to acquire single unit activity and local field potential information, or oscillatory activity. LFP recordings show oscillations representing rhythmic membrane depolarizations. Oscillating membrane depolarizations have been shown to have two main roles, modulation of local inhibition and synchronization of neural activity(Buzsaki et al., 1983). It has also been suggested that these oscillations may serve as a transport vehicle for replay and trajectory events across different brain regions. After all, systems level consolidation is thought to be the results of a dynamic interaction between populations of neurons in both the hippocampus and neocortex.

Electrical activity patterns first observed in the hippocampus were categorized by a behavioral correlate (Figure 18). Vanderwolf, 1969, placed a large electrode into the hippocampus of a freely moving rat during different behavior and identified three states: a rhythmical theta state, a large irregular amplitude activity (LIA) state, and a small amplitude irregular activity (SIA) state(Vanderwolf, 1969). The LIA state is thought to represent a more relaxed state of the network or a state in which memories previously encoded in the hippocampus are strengthened or transferred to other brain regions. This

state is characterized by sharp waves of 100ms with an average interval of 1s, and associated with high ripple oscillations of 100-200Hz. Sharp waves occur during behaviorally quiet periods, such as during slow wave sleep and consummatory behaviors, and are thought to originate in the CA3 and phasically drive CA1 neurons (Buzsaki et al., 1983). Replay and trajectory events take place during sharp wave activity. Both theta and ripple oscillations are thought to reflect inhibitory network activity. Theta ranges from 6 to 12Hz and has been reported at 4Hz in the rabbit. Theta rhythm is found when rats are moving or exploring. Stimulating neurons during theta rhythm have been shown to facilitate the induction of LTP(Mott and Lewis, 1991).

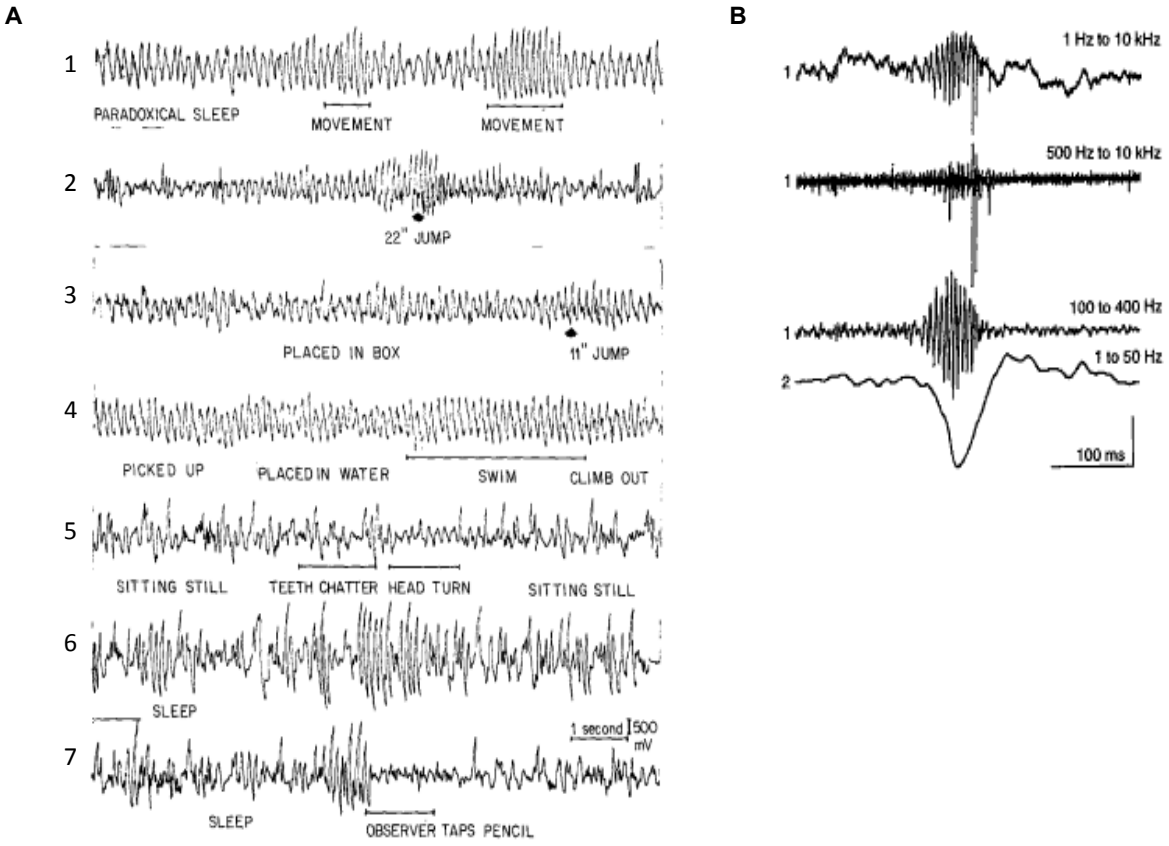


Figure 18. Oscillatory Wave Patterns In The Hippocampus. A, The different electrical patterns of activity in the hippocampus and their correlated behaviors. Theta is observed throughout the different behavioral states; (1) rapid-eye movement (REM) sleep, (2,3) jumping, and swimming (4). During quiet sitting (5) and slow wave sleep (6) is when large irregular amplitude activity takes place and large amplitude sharp waves occur. B, LIA ripples and sharp waves in broad-band (the very top) and filtered at different frequency bands. Recordings from electrode 1 are from CA1 pyramidal layer. Electrode 2 is placed in the stratum radiatum, right in between CA1 and CA3. (Vanderwolf, 1969)

Methods

Analysis of Place Cell Ensemble Activity: Defining Highly Correlated Replays and Trajectory Events

Place cell ensemble activity was explored during stopping periods at the end of every lap. Candidate replay events were identified during periods of increased spike density, with peaks greater than 3 standard deviations above the mean in the smoothed histogram. Start and end boundaries for each candidate replay event were defined where the smoothed histogram crossed the mean. Spiking activity was divided into non-overlapping 20ms time bins and position estimated. In order to quantify replay, a set of thresholds on various measures of sequence quality were used to identify a subset of candidate events as replays, and critically, the same criteria were used to identify replays in subsequent shuffle analyses. Therefore, the number of events passing criteria by chance could be compared with the number actually detected to determine replay significance. Events within a 100-500ms time window, satisfying a weighted correlation between time and decoded position $|r|$ value ≥ 0.5, and a continuity measure requiring the distance between peak decoding position in neighboring time bins less than 75cm in the direction of the propagation of sequence, and less than 37.5cm in the opposite direction, were considered experienced trajectory events (examples in Figure 19). The direction of the sequence was determined by calculating the averaged peak decoding position of the first half and the last half of the sequence, with the first and last time bins excluded. If the first half was 75cm smaller than the other half, it was defined as going in the outward direction; if it was 75cm larger than the second half, it went in the opposite direction, inward. An adapted form of the Pearson's correlation, weighted correlation

measures the strength of correlation between time and position weighted by posterior probability and thus utilizes the entire posterior probability distribution:

Weighted mean: $$m(x;w) = \sum_{i=1}^{M}\sum_{j=1}^{N} w_{ij}x_i \Big/ \sum_{i=1}^{M}\sum_{j=1}^{N} w_{ij}$$

Weighted covariance: $$\mathrm{cov}(x,y;w) = \sum_{i=1}^{M}\sum_{j=1}^{N} w_{ij}(x_i - m(x;w))(y_j - m(y;w)) \Big/ \sum_{i=1}^{M}\sum_{j=1}^{N} w_{ij}$$

Weighted correlation: $$corr(x,y;w) = \mathrm{cov}(x,y;w) / \sqrt{\mathrm{cov}(x,x;w)\mathrm{cov}(y,y;w)}$$

where x_i is the i^{th} time bin, y_j is the j^{th} position bin, w_{ij} is the probability of pixel (i,j), M and N are the total numbers of time and position bins of a given track.

To determine the significance of the identified replays, the number of replays was compared to the distribution of numbers of replays calculated after applying position estimation and replay detection criteria to 5000 shuffled data sets. To generate shuffles, the place field for each cell was translated by a random distance (different for each cell, with wrapping at edges), thereby preserving firing statistics (spike trains were not changed) and largely preserving individual place field structure, but dis-coordinating position information across cells. This is a more conservative measure than randomizing the mapping between fields and spike trains, which does not preserve individual place field structure, and is more conservative than randomizing position or time bins after the decoding has been calculated, since this will artificially benefit from smoothness in the position and/or time domain. Associated Monte-Carlo P-Value of the position correlation scores was calculated and a P-Value below 0.05 was considered significant.

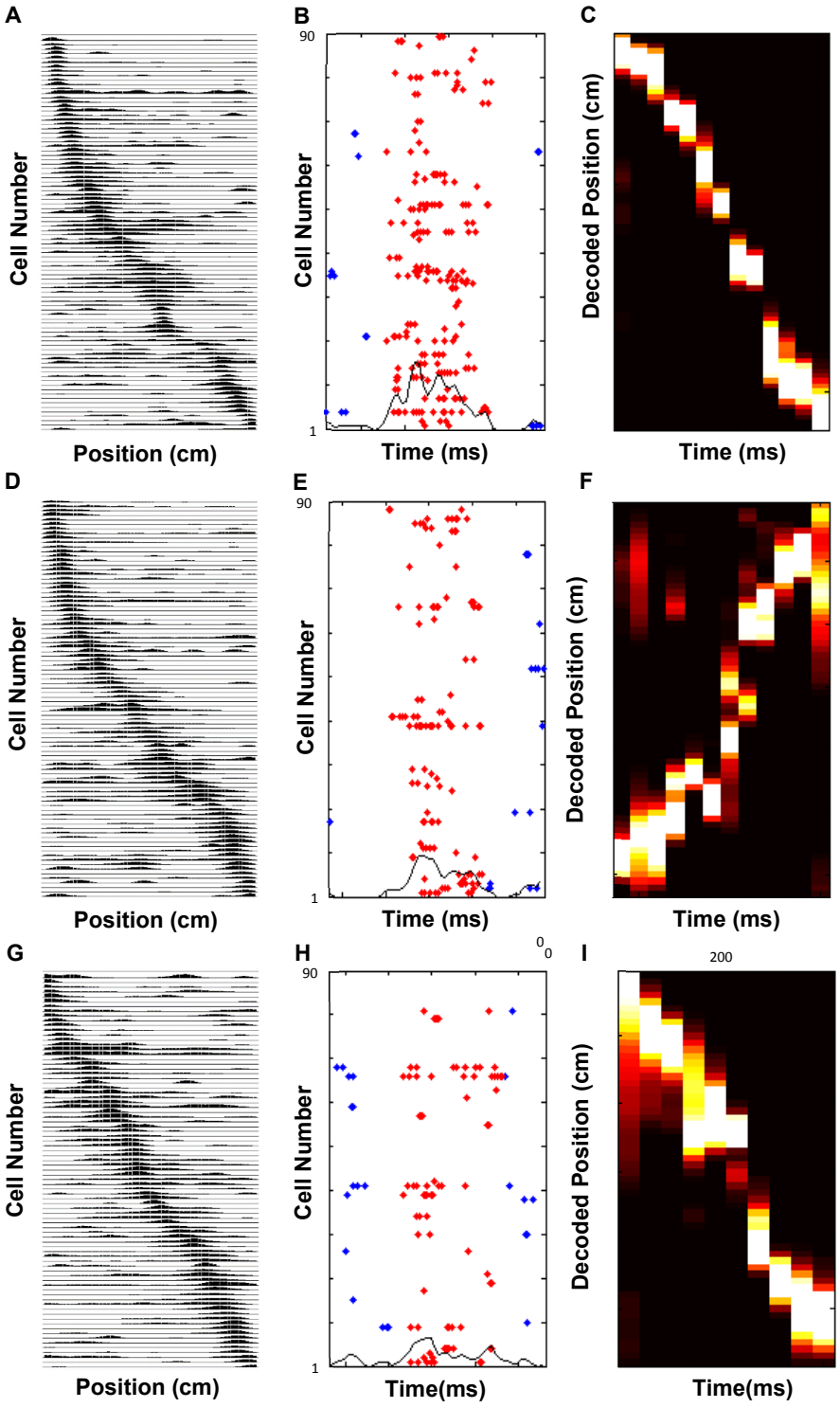


Figure 19. Defining Trajectory Events. A, D, and G are rate maps of place cell ensemble activity. This represents the place cell activity of 90 cells on the track. The raster plots in B, E, and

H take place at the end the track. The spikes in red are those that fall into an increased spike density window, 3 standard deviations above the mean. The spike density is plotted on top of the spikes. This spiking activity divided into non-overlapping 20 ms time bins and position is estimated using a probability based decoding algorithm as is show in C, F, and I. In C for example there are 12 times across, which means this event is 240 ms long. The number of position bins and time bins is different for each event. These events then undergo additional analysis that defines them as trajectory events. The three examples here are trajectory events because they all have a weighted correlation between time and decoded position of |r| > 0.5 (time and decoded position), the pass a continuity measure (explained in the Methods section), and fall within a 100-500ms time window.

Results

Experience Gives Structure to Place Cell Synchronous Activity

Every experiment was designed to begin with a pre-sleep session, Sleep1. The day of the recordings the animals have had no running experiences prior to the beginning of Sleep1, and the three tracks which they are about to experience that day are completely novel. To determine whether any form of replay existed in Sleep1 prior to experience, we estimated position for candidate events using place fields obtained from Runs1, 2 and 3. Figure 20 shows candidate events of Track1 replays with a weighted correlation greater than 0.5 are found in Sleep1 replicating that a firing structure can be found when measured against a background null hypothesis. The figure re-illustrates the experimental design as well the selectivity of the places fields for each track during the different runs. The candidate events or preplays in Figure 20C were identified using the place fields diagrammed in Figure 20B. The results of the group data for all three runs are shown in the shuffle plots. An important observation which Dragoi et al. also mentioned is the increase in the number of events that occur during the runs compared to Sleep1.

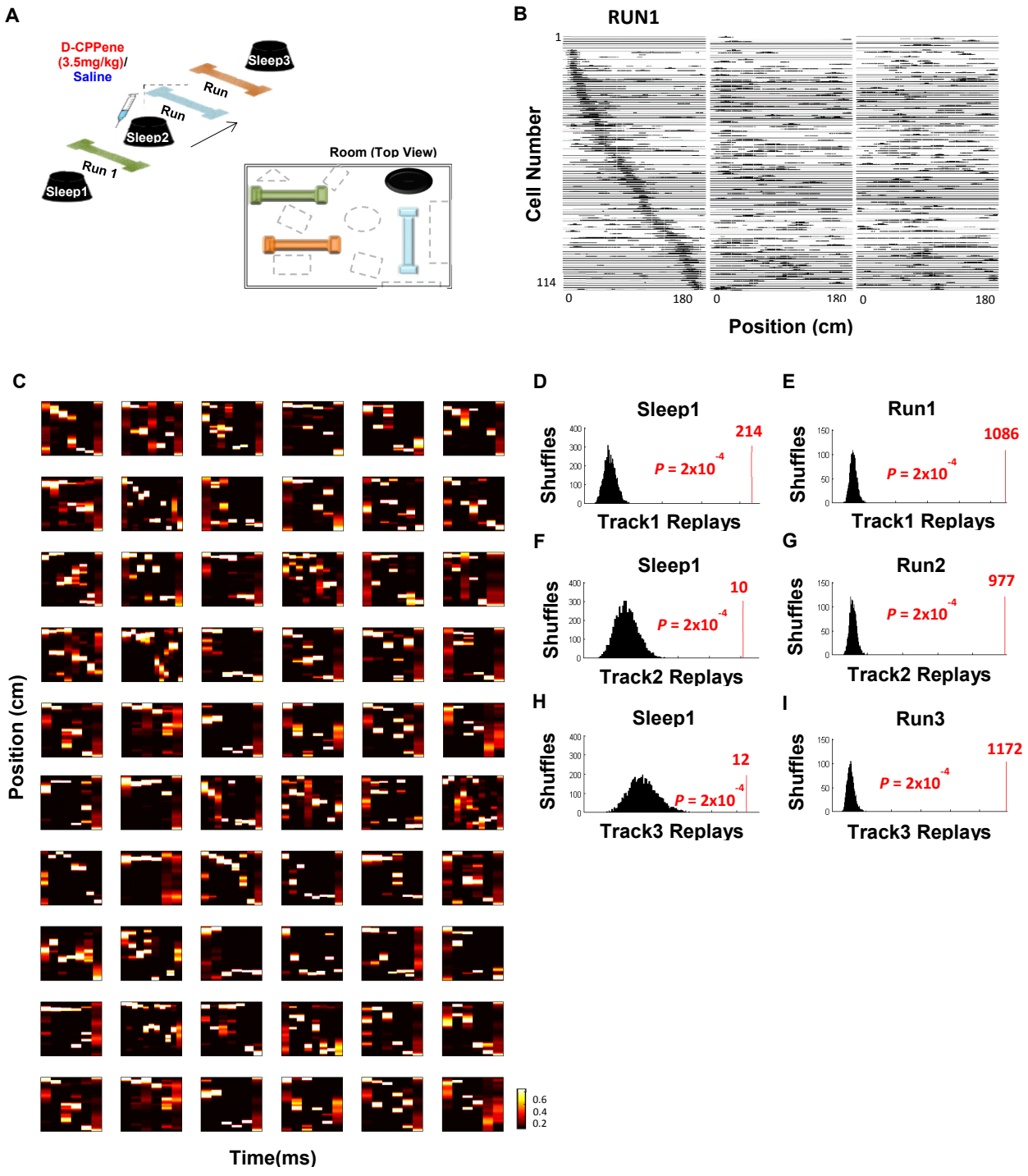


Figure 20. Highly Correlated Events in Sleep1. A, Experimental Design. B, Rate maps of 114 place cells for one animal arranged by the peak firing rates during Run1, used to identify events in Sleep 1 in C, with a correlation greater than 0.5 and lasting between 100-500ms. D-I, Histograms of shuffled group data for each track during Runs and in Sleep1. Numbers of highly correlated events detected in each actual dataset are indicated in red. Monte-Carlo P-value shown for each Run and Sleep condition.

When we looked at candidate events after experience (Figure 21), those identified while animals were running on the different tracks, we observed that these events although also highly correlated were also much more structured. These results suggest that weighted correlation between time and decoded position alone does not illustrate or capture the structure of sequence activity or the continuous movement found in replays during run. To try and differentiate structured replay from highly correlated replays or preplays we included additional parameters described in the methods. In particular, we applied a continuity measure that depicts the continuous movement of the trajectory the animals have experienced. These replays found during the runs we term trajectory events. When we try to look for trajectory events of the different runs in Sleep1, the group data reveal that there are very few events and none of the identified events are significant above chance (Figure 21B,C,D). This finding suggests that experience does change the organization and structure of sequence activity during replay to reflect the trajectory or experience just had.

The term reactivation when used in the literature does not always distinguish between the reactivation of place cells that represent a continuous trajectory or a sequence and those that do not. O'Neill and Csicsvari's work points at a difference in plasticity mechanisms for reactivation of places versus reactivation of sequences, or reactivation with a temporal structure(O'Neill et al., 2006). Place reactivation was defined as coactivity within a 100ms time period, a time period which is long enough for reactivation events with a sequence or temporal structure like replays.

It is important to note that although we report an absence of novel trajectory events and spatial coordination prior to experience, this may not necessarily be the case in a familiar environment. Data from our lab (Pfeiffer and Foster, 2013)and others (Gupta et al., 2010), describe replays of sequences or trajectories that have never been experienced before, reflecting novel combinations of familiar experiences or trajectories. We suggest these replays would be independent of experience-dependent forms of plasticity. A key behavioral difference in our current study is that we are exploring the initial learning of a completely novel environment, which is very different than learning novel goal locations in a familiar or already learned environment.

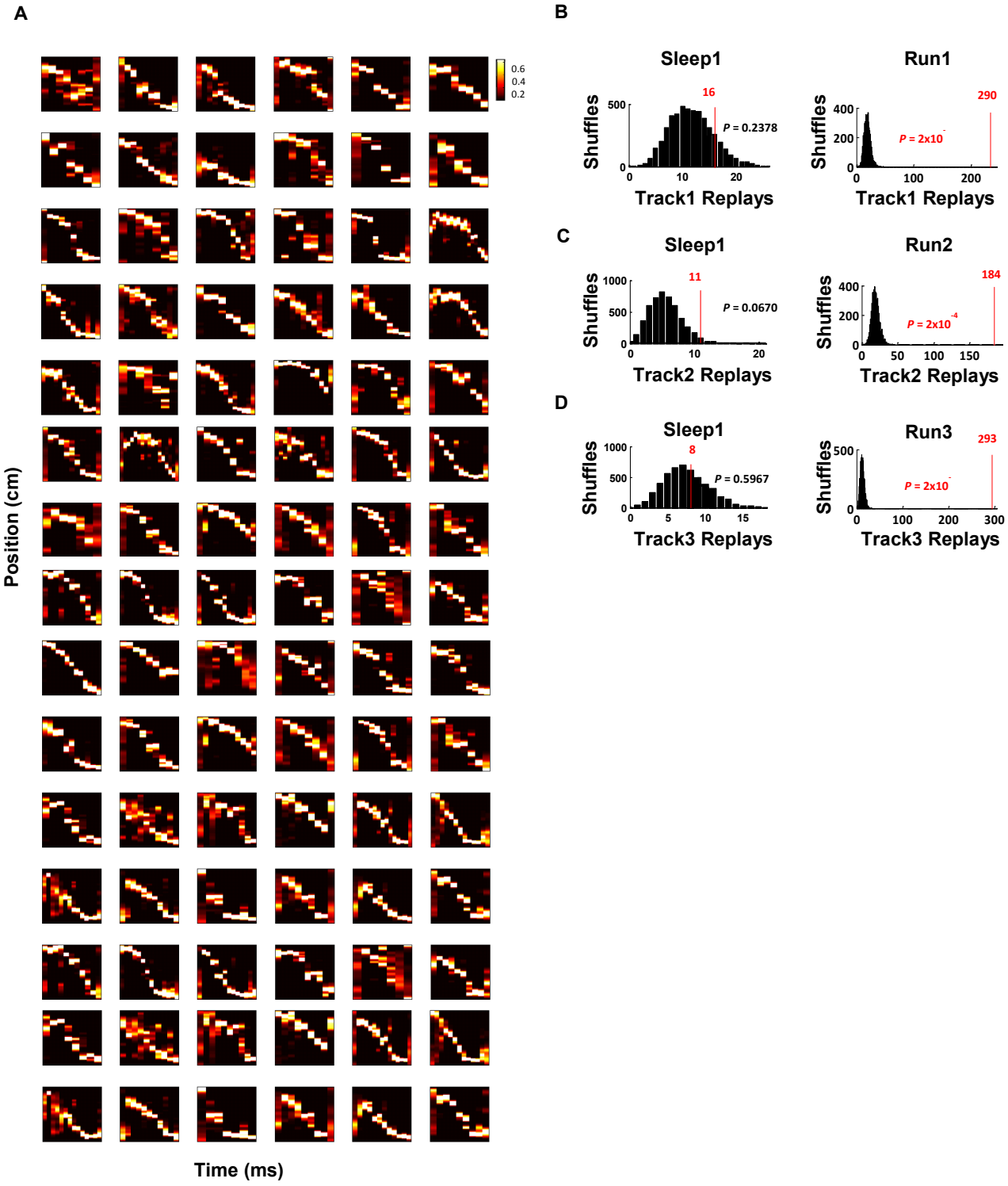


Figure 21. Trajectory Events Found Only After Running on the Tracks. A, Trajectory events identified during Run 1 using the same 114 place fields as in Fig. 20 (B). B-D, Histograms of shuffled group data for each trajectory event during each Run and in Sleep1. Numbers of trajectory events detected in each actual dataset are indicated in red. Monte-Carlo P-value shown for each Run and Sleep condition.

Encoding of Trajectory Events Requires NMDA Receptor Function

We investigated the role of NMDAR function in the encoding of a memory trace for explored trajectories, expressed as sequential replay or trajectory events. We learned from work of others a range of times for which NMDAR function is necessary for spatial learning and purposely built in multiple novel experiences within one recording day into our behavioral design(Morris et al., 1986; Morris et al., 2013). We know that place cells remap between experiences and environments. If ensemble activity is constantly remapping then the memory of a particular experience may depend on its replay pattern. According to Foster et al., 2006, there is no correlation between the replay of a familiar track and a novel one. We can then postulate that trajectory events may serve as a stable representation of memory or a memory trace. By blocking NMDA receptors pharmacologically we are able to further test the experience-dependency of trajectory events. The hypothesis is that the experience on the track may lead to changes in synaptic weights which are modulated by synaptic plasticity mechanisms that may be perturbed under NMDAR antagonism.

We examined awake replays occurring during the periods of exploration on the three novel tracks: Runs 1, 2 and 3 (Figure 22). Saline group data is plotted in Figure 21B-D. During Run 1, which was prior to the injection of drug or saline, highly significant replay trajectory events were observed for both saline and D-CPPene conditions, as expected (Figure 21B and Figure22A). After injection with saline, highly significant trajectory events continued to be observed during Runs 2 and 3 (Figure 21C and D). Strikingly however, trajectory events were completely absent under D-CPPene

during Runs 2 and 3. The best correlated events lacked any apparent structure (Figure 22B and C). Comparison with the shuffle distribution revealed that under D-CPPene numbers of sequences were indistinguishable from chance. Thus, in the presence of D-CPPene in Runs 2 and 3, trajectory events were not observed, indicating that despite relatively normal position coding during behavior (Chpt2), place cells were unable to organize in a sequential pattern reflecting the spatial memory of the running trajectory. In Figure 22, the red box events are under the effects of NMDAR antagonism. Here, the best correlated events were chosen across all group data. The lack of structure or absence of trajectory events under NMDAR antagonism can also be detected if we were to look at all the candidate events from one D-CPPene recording session and compare that to the candidate events from a saline section (Appendix D and E). In these figures there is a striking difference between the saline and D-CPPene events that illustrates that under D-CPPene the learned structured is not acquired.

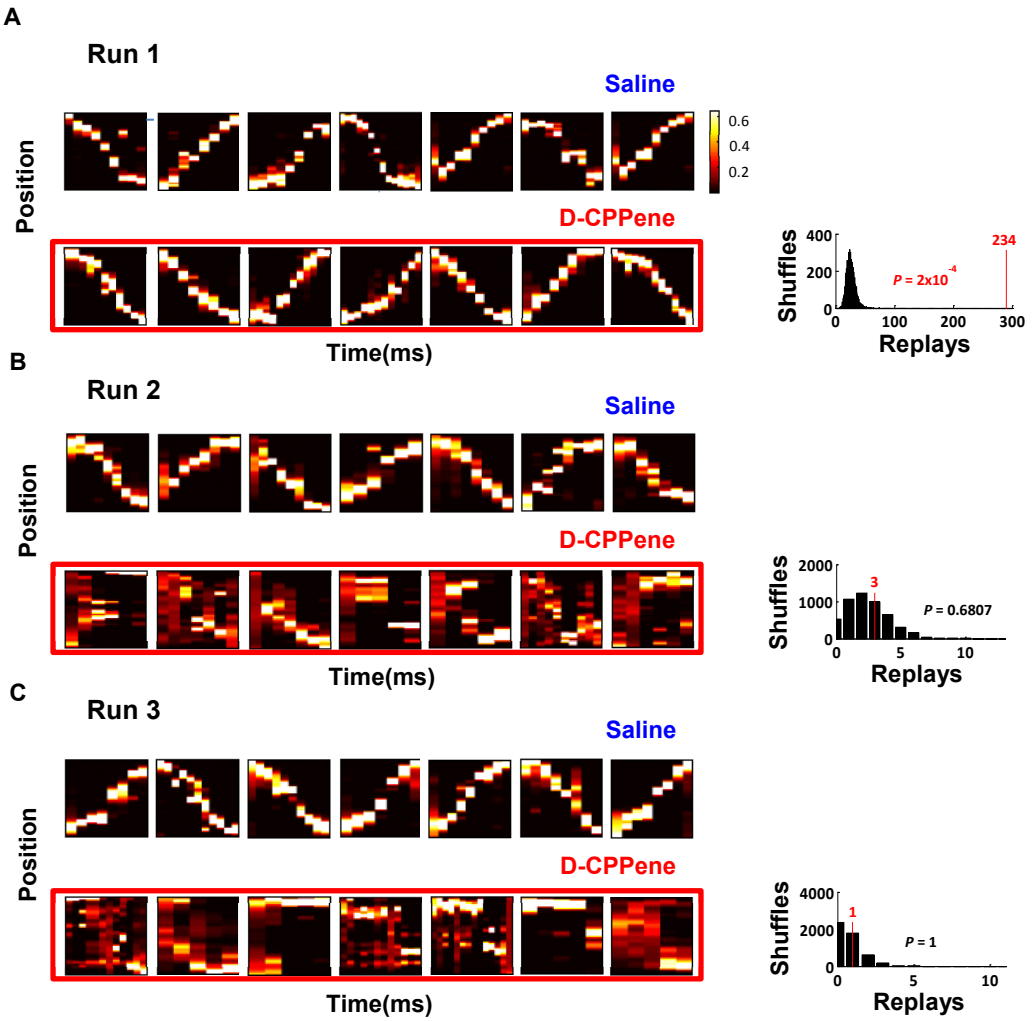


Figure 22. NMDA Receptor Function Is Necessary for the Acquisition of Trajectory Events. A, B, and C, Selected trajectory events expressed during Run 1, Run 2 and Run 3 on saline (top) and D-CPPene (bottom) days. Shown are the seven highest correlated candidate events for each run. To the right are histograms of shuffled D-CPPene data for each Run. Numbers of detected trajectory events in each actual data are indicated in red. Monte-Carlo P-value shown for each Run and condition. Shuffle results for saline Run data already shown in Figure 21 B-D.

Place Field Activity and Replay Activity May be Mechanistically Distinct

Under NMDAR antagonism we have demonstrated that even though place fields have subtle effects, replay is completely abolished. These findings suggest that mechanistically place field firing and replay may be distinct. It's been demonstrated that replay is more readily observable, or more robust, in a novel track (Cheng and Frank, 2008). This is interesting as it is contrary to that observed when looking at place fields; place fields are less stable in a novel environment (Wilson and McNaughton, 1993). To determine if the apparent impairment in memory or replay encoding under NMDAR antagonism could be explained by the relatively subtle changes in place fields under drug, we repeated the analysis of the saline-treated animals using only the subset of the recorded neurons which had similarly broad and diffuse place fields to those recorded under D-CPPene treatment. Decoding of saline runs using only place cells with diffuse fields yielded position estimation significantly greater than chance (Figure 3B-D). Critically, clear replay sequences were observed when only using diffuse place fields in the analysis, and the numbers of sequences observed were highly significant compared to chance (Figure 23 E-J). These results indicate that the changes to place fields observed under NMDAR antagonism could not by themselves account for the observed effects of NMDAR antagonism on replay.

Steele et al., 1999, showed NMDARs in hippocampus are critical for animals to learn from a single trial if they are to hold on to that information for more than a few seconds. The exact time at which memory would disappear without NMDAR dependent encoding is unknown but the two time points tested in their study were 15s and 20 min. Place fields may undergo subtle changes but more importantly they persist over these

time periods. Kentros et al., showed place fields could form under NMDAR antagonism and speculated that the critical period of degradation would be at 16-24hrs, which they defined as a period of long-term stability. This suggests that replay encoding is a better model of the neural mechanism for one-trial spatial learning.

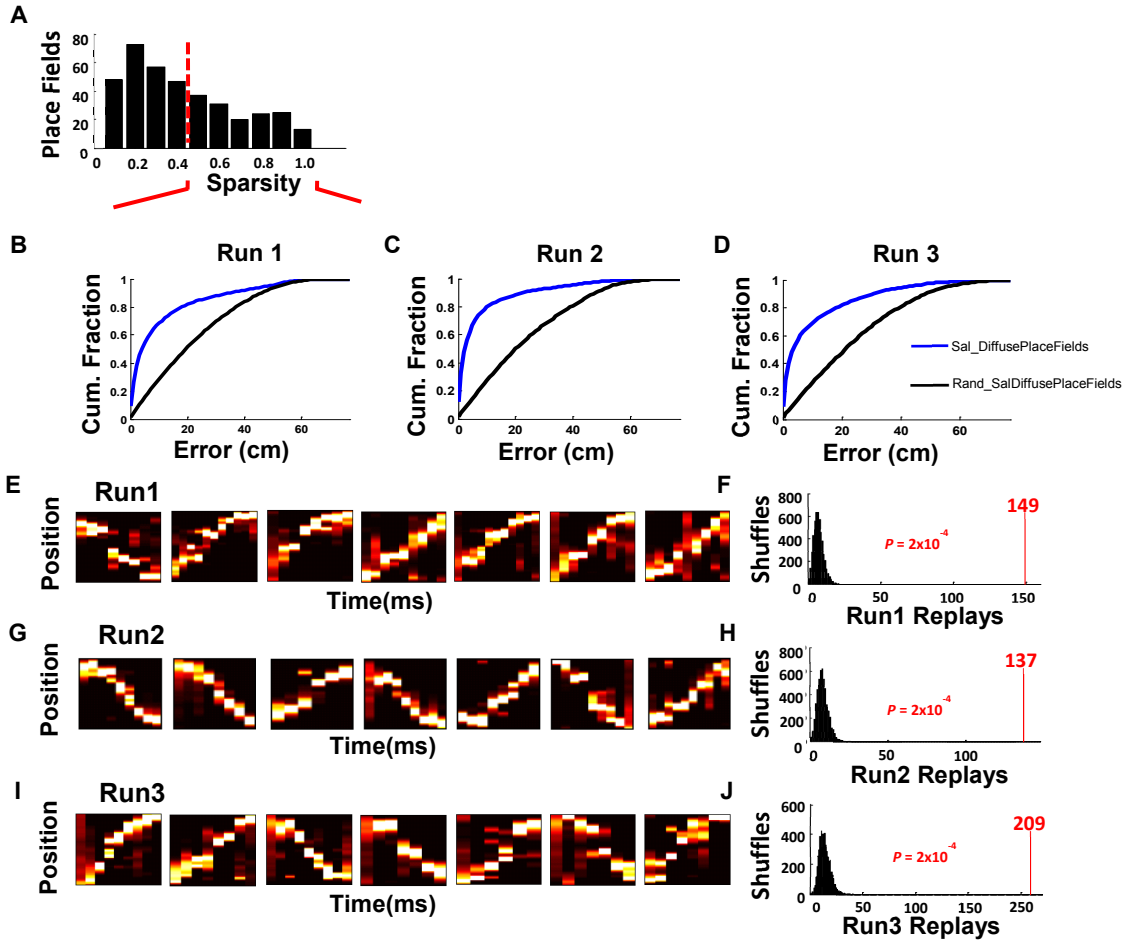


Figure 23. Diffuse Place Fields Do Not Increase Run Decoding Error and Do Not Impair Trajectory Events. A, Model histogram of place field sparsities from saline Run . B-D, Position decoding using only the subset of place fields with sparsity value greater than 0.5, Sal_DiffusePlaceFields in each Run. E, G, and I, Selected trajectory events expressed during saline Runs using only diffuse place fields. F, H, and J, Corresponding shuffle analysis for each case.

Sharp Wave Ripples Appear Unaffected by NMDAR Antagonism

In both saline and D-CPPene group data, candidate events co-occurred in all three Runs with sharp-wave ripple (SWR) events in the hippocampal local field potential (Figure 24). Thus the events we found were confirmed as functionally similar to SWR-associated place-cell sequences that have been reported many times previously(Diba and Buzsaki, 2007; Foster and Wilson, 2006; Gupta et al., 2010; Karlsson and Frank, 2009; Lee and Wilson, 2002; Pfeiffer and Foster, 2013). We therefore asked whether the abolition of sequence information under D-CPPene was accompanied by changes to the structure of the associated SWRs, since such changes would shed light on the mechanisms responsible for generating replay sequences. In particular, some models of hippocampal sequence production predict that synaptic plasticity should contribute to sequence generation by coordinating the propagation of excitation between successively active ensembles of neurons at each time step (Gewaltig et al., 2001; Tsodyks et al., 1996). Such models would predict that, in the absence of synaptic plasticity, sustained sequences should not occur, leading to truncated population activity and hence truncated SWRs.

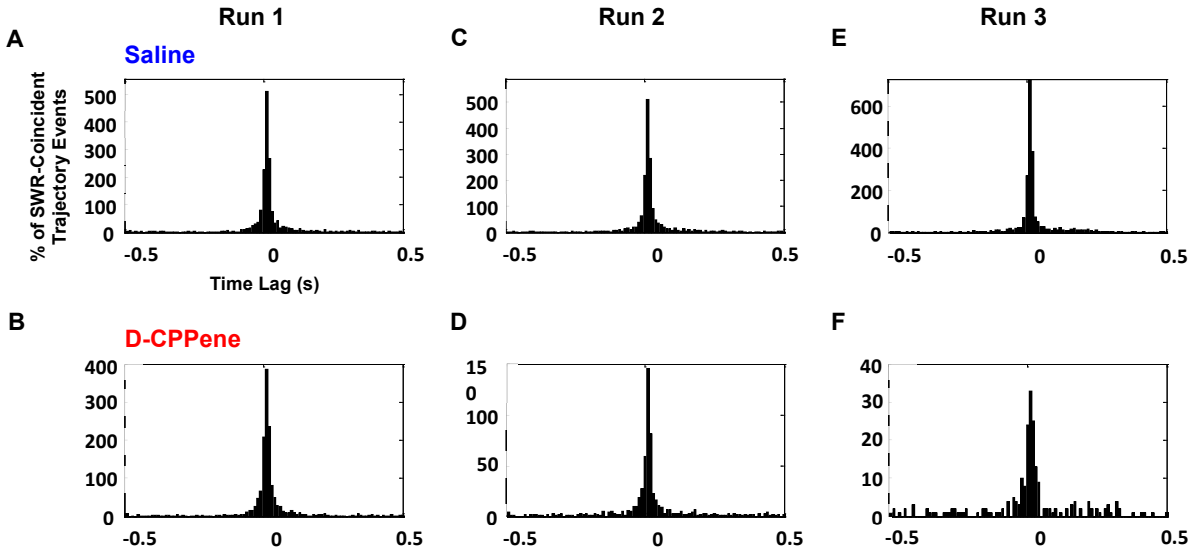


Figure 24. Cross-correlation Between Trajectory Events and Ripple Occurrence. Cross-correlograms for candidate events with ripples across all Runs, for each condition, saline, A, C and E, and D-CPPene, B, D and F.

In order to test the hypothesis that plasticity is necessary for the sustained production of population events, we examined the amplitude, duration and rate of occurrence of SWRs either under drug or under saline, in the three runs. For each tetrode, one representative channel was selected and the LFP signal was analyzed. The LFP was bandpass filtered between 150 and 300 Hz, and the absolute value of the Hilbert transform of this filtered signal was then smoothed (Gaussian kernel, SD = 12.5 ms). This processed signal was averaged across all tetrodes and ripple events were identified as local peaks with an amplitude greater than 3 SD above the mean, using only periods when the rat's velocity was less than 5 cm/sec. The start and end boundaries for each event were defined as the point when the signal crossed the mean.

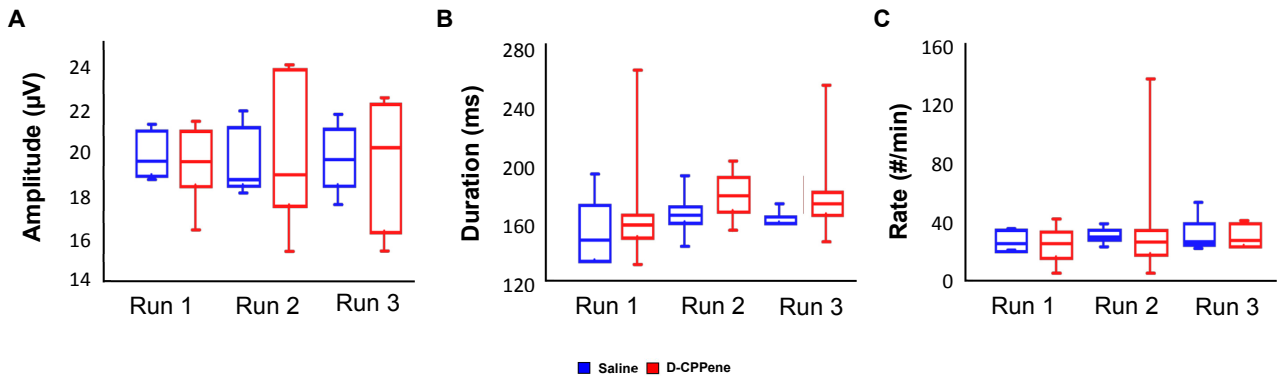


Figure 25. Ripple Event Analysis. A, Amplitude, B, duration, and C, rate of ripples for each Run. *P*-values (Wilcoxon rank-sum test): Saline vs. D-CPPene amplitude, Run 1, 0.9740, Run 2, 0.8182, Run 3, 0.8182; duration, Run 1, 0.8182, Run 2, 0.2403, Run 3, 0.1320; rate, Run 1, 0.5887, Run 2, 0.3939, Run 3, 0.8182.

Surprisingly, there was no difference between conditions in SWR amplitude, or in SWR duration, in any of the runs (Figure 25 A,B). There was also no significant difference in rate of occurrence of SWRs (Figure 25C). These data suggest that, in contrast to the predictions of some models of sequence generation, inherent oscillatory activity may be unaffected by NMDAR antagonism and therefore that sequentially structured patterns of co-activity may not be necessary for the sustained transmission of neural spiking during SWR. Rather, the production of SWRs may be a highly controlled process, for example by interneuron populations(Klausberger and Somogyi, 2008), that is robust against degradation in the associated information content. It remains unknown, therefore, exactly how replay sequences might be generated, and also exactly which circuits play a critical role(Buzsaki, 1989; Lisman et al., 2005).

Chapter 4: Replay Retrieval

Introduction

Neurons that fire together during awake behavior tend to fire together again during sleep (Wilson and McNaughton, 1994). Replay occurs during sharp wave ripple complexes which are found during quietness and slow-wave sleep. These oscillations have been proposed to provide the substrate to promote consolidation of memory traces. Increase in ripples or ripple occurrence has been associated with predicted learning and an increase in performance in spatial memory tasks (Eschenko et al., 2008; Ramadan et al., 2009).Place cells that fire together when an animal occupies a particular location in an environment exhibit an increased tendency to fire together during subsequent sleep(Wilson and McNaughton, 1994). Repetitive experience of a particular spatial sequence results in a memory trace of that sequence during slow wave sleep(Lee and Wilson, 2002). Reverse replay observed during stopping periods after an animal runs on a track point towards a mechanism by which the encoding of a memory begins. Replay may serve as a transient trace which is consolidated during sleep.

Retrieved replay in sleep is not very well characterized. The hippocampus is just one brain region involved in memory retrieval. Human and monkey recordings and imaging studies have provided evidence for the frontal regions, the hippocampus, and the amygdala being activated during memory retrieval (Spiers and Maguire, 2007; Wheeler and Buckner, 2004). Where retrieved replay originates, how long it is present in the hippocampus, and how it is transferred to other brain regions still remain unanswered questions in the field. It is then imperative, to understand how the sequence play

observed at stopping periods relates to the retrieved replay found during sleep or periods of quiet rest.

Methods (see Chpt 3)

Results

Retrieval of Replay Sequences is Not NMDAR Dependent

It is a long-standing finding that while NMDA receptor blockade can prevent the formation of long-term potentiation and the establishment of memory, such manipulations spare established plasticity or memory(Bannerman et al., 1995; Collingridge et al., 1983; Morris, 1989; Saucier and Cain, 1995). To determine whether a similar distinction exists on the effects of NMDA receptor antagonism on replay sequences, we examined decoded position during candidate events occurring in Sleep 3, a period of sleep/quiet rest, using spike trains and place fields obtained from Runs 1, 2 or 3. In this way we quantified the occurrence of replays of Runs 1, 2 or 3 during Sleep 3, while NMDAR dependent plasticity remained blocked by D-CPPene (Figure 26).

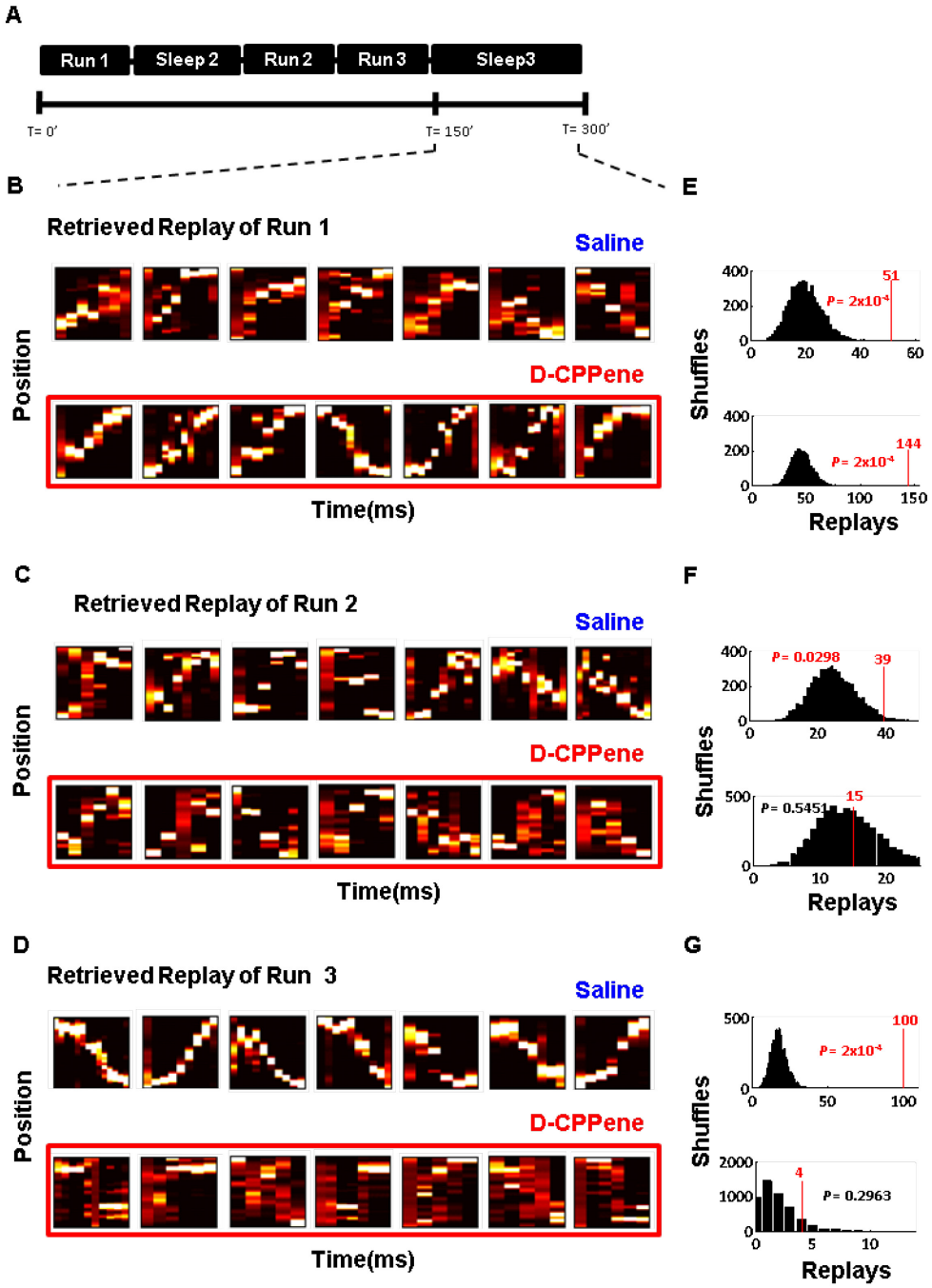


Figure 26. NMDA Receptor Function is not Required in Replay Retrieval. A, Experimental timeline. Sleep 3 begins at an average of 2.5hrs into the recordings and continues for another 2.5hrs. B,C and D, Replays of Run 1, Run 2, and Run 3 during Sleep 3 under saline (top) and D-CPPene (bottom). Shown are the seven highest correlated candidate events in each case. E, F and G, Histograms of shuffled data. Numbers of replays and associated Monte-Carlo P-Value, indicated in red in each case when significant.

Under saline, significant numbers of replays were observed in Sleep 3 for all three prior experiences. Under D-CPPene, consistent with the earlier results for awake replay, replay was not observed in Sleep 3 for either post-injection Runs (i.e. Runs 2 and 3; Figure 26 C,D,F,G, lower panels). However, in striking contrast to these results, clear replay under D-CPPene was observed of Run 1 in Sleep 3 (Figure 26B, lower panel). The number of replays of Run 1 in Sleep 3 was highly significant compared to shuffles. Thus, strong replay was observed of behavioral episodes experienced prior to the blockade of NMDAR-dependent plasticity. Taken together, these data demonstrate that NMDAR antagonism does not affect the retrieval of memory in the form of replay sequences once memory has already been established. Rather, NMDAR antagonism selectively blocks the establishment of memory encoding during behavior, which is necessary for the subsequent expression of that memory in the form of replay sequences. To look at more trajectory events in sleep 3, see Appendix F and G.

The distinction between encoding and retrieval is further illustrated by examining the 15 minutes of sleep immediately preceding Run 2, or the 15 minutes immediately following Run 3 (Figure 27). Each of these periods was immediately proximate to a period during which the presence of D-CPPene prevented the encoding of new memory. For example, no replay was observed in Run 2 under D-CPPene , by contrast during the 15 minutes immediately preceding, significant numbers of replays of Run 1 were observed (Figure 27B). Likewise, whereas no replay was observed during Run 3 under D-CPPene, by contrast during the 15 minutes immediately following, significant numbers of replays of Run 1 were observed (Figure 27C). Thus, the abolition of replay was highly selective even within similar time periods, with a requirement for NMDAR activation in

the encoding of sequence memories but not for the retrieval of sequence memories that had already been encoded.

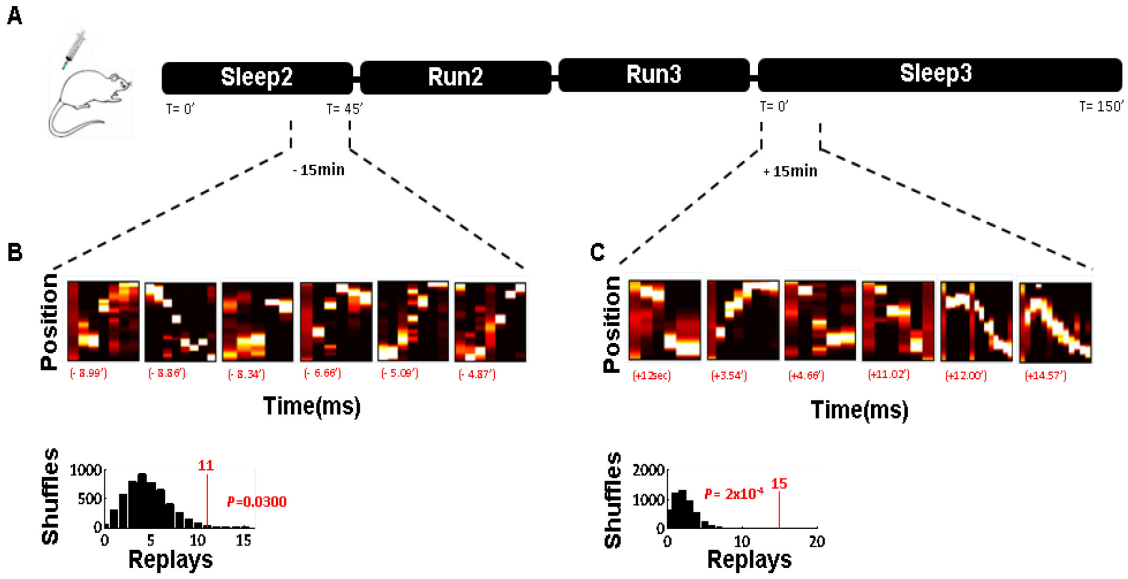


Figure 27. NMDAR Antagonism Prevents Expression of Novel Replays Throughout the Length of the Experimental Session. A, The timeline shown indicates that when looking at the last 15 minutes of Sleep2, B, and the first 15 minutes of Sleep3, C, significant replays of Run 1 are retrieved post administration of D-CPPene and are expressed minutes before rats experience Run 2 and continue to be retrieved seconds to minutes after the completion of Run 3, indicating that D-CPPene is active at the beginning of Run 2 and is still active at the end of Run 3 and into the beginning of Sleep3. Timing of events are indicated below each replay event along with histograms of shuffled data for the demonstrated 15 minute window.

Decay in the Persistence of Retrieved Replay May Reflect More Than Just the Passage of Time

It has been proposed that hippocampal place cell reactivation decays or degrades with time, with early reports based on pairwise measures of reactivation strength estimating a decay constant of only minutes (Wilson and McNaughton, 1994). We were able to replicate the pair wise results (Appendix H). We monitored replay events of the latest experience, Run 3, in saline animals (Figure 28). By contrast, we found that replay of sequences persisted without apparent degradation throughout a period of at least three hours subsequent to the end of the behavioral session. Although, the actual replay numbers are not enough to test statistical significance we note that neither an increasing or decreasing trend was observed. However, when considering all candidate events we also found there was no decrease in expression of candidate sequence activity. Hence we find no basis for expecting this replay to decline at a later point. We did however find that replay of the most recently experienced track was much stronger than that of tracks experienced earlier in the day (Figure 29). In other words, in control conditions, of all the retrieved replays expressed during Sleep3, retrieved replays of Run 3 were the most prominent.

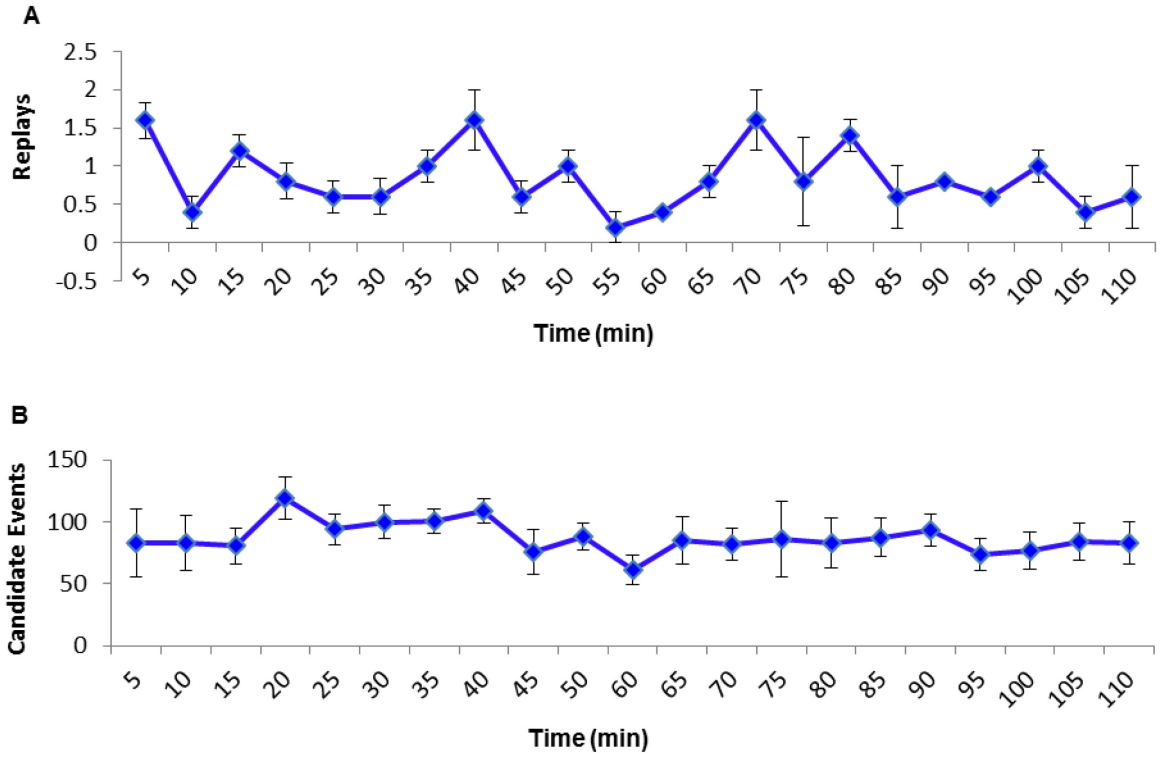


Figure 28. Rate of Run3 Retrieved Replays and Candidate Events Over the Duration of Sleep3. A, Replay rate (no. replays/5min) and, B, number of candidate events of retrieved replays of Run3 throughout 110min in Sleep3. Plotted is the saline/control group data (mean ±SEM).

We considered two alternative hypotheses for why the rate of replay should be reduced for Runs 1 and 2 during Sleep 3 when compared to Run 3. This could reflect an automatic decay of replay with elapsed time, as previously proposed on the basis of pairwise reactivation studies(Kudrimoti et al., 1999; Wilson and McNaughton, 1994). Alternatively, competition between replay of the different experiences could occur, in order to favor the later Run period. In order to distinguish between these two hypotheses, we considered the incidence of Run 3 replay during Sleep 3. This sleep period extended for at least 2.5 hours on each day, and without the possibility of competition from replays relating to intervening experiences. Notably, we observed no degradation in either the

numbers of replay (Figure 29), or in their quality as measured by correlation (Appendix I), or in their duration (Appendix J). Thus, the degradation in replay of Runs 1 and 2 in Sleep 3 was likely due to competition between replays of different experiences. This competition favored the most recent experiences, either transiently by selecting for recent experiences, or permanently by overwriting earlier experiences. This finding is further corroborated by our observation that under D-CPPene, replay of Run 1 was maintained without degradation through the even longer period of 5 hours (data not show beyond time in Figure 29A). In the case of D-CPPene, results can be interpreted as though animals are unable to recall or retrieve anything other than replays of Run1 because encoding of replay was blocked during Runs2 and 3, validating our theory that in the absence of competing experiences, or replays, the last experience will continue to be retrieved without decay.

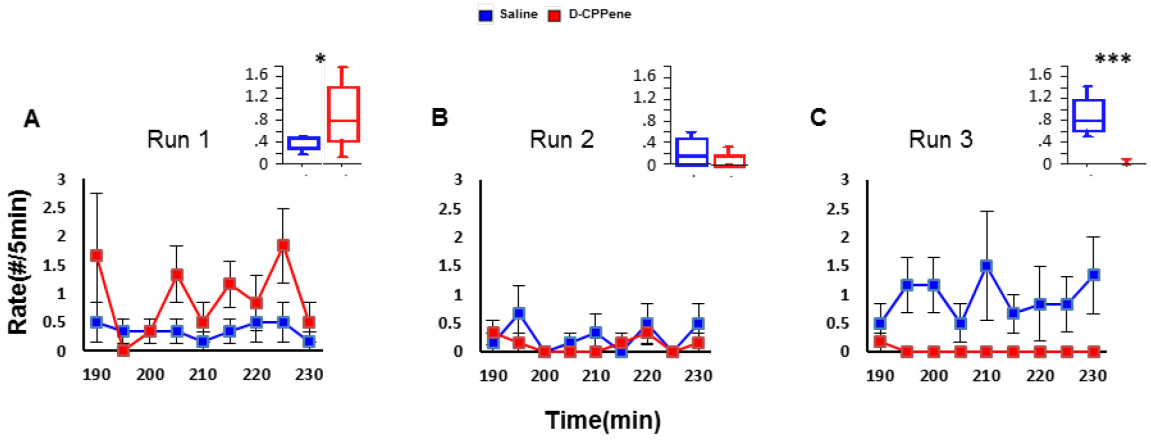


Figure 29. Rate of Retrieved Replays Over Time. A,B, and C, Rate of Run 1, Run 2, and Run 3 retrieved replay in an overlapping 40 min period during Sleep3 across all animals and corresponding box plots illustrating number of retrieved replays in a 5min period. *P*-values (Wilcoxon rank-sum test) (A),*, 0.03101, (C), *, 4.11 x 10^{-5}.**

Taken together, these data suggest that it is not the passage of time *per se*, but rather some form of competition between different memories that leads to a reduction in the strength of replay of earlier experiences. Further experimentation is needed to investigate if under control conditions replay would continue to be retrieved in the absence of competitive replays.

Chapter 5: Future Work

Our results provide strong evidence that plasticity and experience is necessary for the encoding of replays of a novel environment. The use of NMDAR antagonism proved to be advantageous because it allowed us to separate the two phases of memory processing, encoding and retrieval. We proposed neural mechanisms that may mediate spatial memory encoding and retrieval. However, we have not yet identified what specific molecular mechanisms are necessary. By using an NMDAR antagonist that was administered globally, we also did not specify what other brain regions in addition to the hippocampus may be essential during encoding and retrieval. Other topics which we touched on worth further experimentation include place cell stability and the persistence of replay or memory traces. Additional behavioral experiments would also help clarify and broaden the significance of the work and findings presented in this dissertation.

Neuromodulatory Mechanisms to Explore

We hypothesized that reverse replay may reflect a pattern of increased synaptic strengths distributed across groups of neurons necessary for spatial memory formation. By disrupting NMDAR dependent plasticity, we were able to disrupt the encoding of replay. However, there are other plasticity mechanisms that may affect synaptic strengths within the hippocampus, such as neuromodulators, dopamine and β-adrenergic signaling.

The hippocampus receives dopaminergic projections from the mesolimbic structures, ventral tegmental area and substantia nigra (Lisman and Grace, 2005). The firing rates of VTA dopaminergic neurons are increased by exposure to novel stimuli(Levy and Steward, 1983). Dopamine (DA) neurons are also activated during the

expectation and receipt of positive reward. The novelty and reward associated firing of dopaminergic VTA could lead to dopamine release in the hippocampus. D1 receptor activation also increases the excitability of interneurons and GABAergic conductances which may play a role in regulating synchronous and oscillatory activity of large populations of pyramidal cells(Lisman and Grace, 2005). Intra-hippocampal injection of D1 and D2 agonists improves performance in the eight arm radial maze(Packard and White, 1991). Exposure to a novel environment facilitates induction of CA1 LTP and is dependent on D1/D5 receptor signaling(Li et al., 2003). Thus, an interesting possibility is that replay could be more robust in the novel track due to DA signaling. The reward at the end of the track may lead to hippocampal DA release and activation of D1/D5 receptors, in conjunction with glutamatergic activation of NMDA receptors. Thus, increase in DA during running in novel tracks may lead to a more robust replay.

Bilateral hippocampal infusion of D1/D5 receptor antagonist, SCH23990 prior to the first trial in the DMP version of the watermaze task resulted in a block of memory retrieval after a 6h interval(O'Carroll et al., 2006). We would use antagonist SCH23990 to determine the role of DA in replay. However, given its transient effects, the timeframe of our experimental design may not be long enough to determine the effects of DA. However, if DA is involved in the formation or expansion of place fields, our experiment paradigm will allow us to see changes.

The locus coeruleus neurons, the source of noradrenaline innervations to the hippocampus, are tonically inhibited during slow wave sleep but show activity prior to waking(Lemon et al., 2009). Activation of β-adrenergic receptors has a significant effect on synaptic plasticity at hippocampal CA1 synapses, mossy fiber synapses, perforant

synapses of the dentate gyrus, and visual cortex synapses(Swanson-Park et al., 1999). Retrograde memory enhancement with systemically administered adrenergic agonists has been found in many experimental tasks and species (even in humans) and interpreted as the adrenergic system being involved with memory consolidation. Previous work on β-adrenergic modulation and memory has been inconclusive due to contradictory results and experimental differences in task and drug delivery. Propanolol injections directly into the amygdala have been reported to cause retrograde amnesia in one trial avoidance tasks(Gallagher et al., 1977). In the MWM task, systemic administration of propanolol immediately after training in water maze reveals impaired memory tested 24h later(Khakpour-Taleghani et al., 2009). β-adrenoceptor activation has been shown to lower threshold for LTP and LTD induction at Schaffer Collateral-CA1 synapses and increase the temporal window for spike-timing-dependent LTP. To determine if β-adrenergic neuromodulation is involved in aspects of replay, propanolol would be used to block β-adrenergic receptors and assess if replay is disrupted. Preliminary data indicates that 5mg/kg propanolol has no obvious effects on place cell activity in a novel environment. Perhaps a higher dose is required to see effects. However, its effects on retrieval of familiar environments and retrieval of both novel and familiar experiences, has yet to be tested with the current dose.

Two different antagonists to test different synaptic plasticity mechanisms would be used in the same experimental paradigm described in this dissertation. Both antagonists would also be administered i.p. The limits of this design would again be the inability to pinpoint exactly which brain regions are involved. However, if we were to locally infuse the antagonists we would not have the ability to look at same cells prior to

injection and after. The local infusion into the brain would cause intraventricular pressure or force that would cause the brain tissue to move and tetrode recordings to become unstable. We have considered the use of transgenic mice, however, the disadvantage of using mice is that replay has not been very well characterized or described in mice. The advantage of the rat is that we can record from a greater number of cells simultaneously and they are also a better model to study spatial behavior.

Behavioral Experiments

Our results indicate that in the absence of NMDA receptor function, replay is not encoded. However, we do not know how this translates to learning directly. In order to test how the absence of replay affects spatial learning we would apply a spatial learning task, such as the three arm T-maze in which rats would have to learn to alternate between arms to receive a reward. We would use trained and non-trained animals. In the trained group, we would train them on the alternation task, administer NMDAR antagonist D-CPPene, and then have the rats perform the alternation task. Given our results so far, we know that D-CPPene does not block the retrieval of replays of prior experiences, therefore we would predict that animals would still be able to perform the alternation task. However, in the untrained group, we hypothesize that without the ability to encode replays, the rats would not be able to perform the alternation task without prior training.

Our work also addressed the persistence of replay. Whether memory traces are temporary or are stored is a topic of interest. It is thought that the hippocampus acts as the integrator of information from multiple brain regions and does not store long term memory. Not all events are remembered, and some events are remembered for longer periods than others. Our attempt to study memory storage and persistence of memory

was done by looking at replay in the final sleep of our experiment. We concluded that when there are multiple replays or competitive experiences to retrieve the most recent memory is the more abundant in the hippocampus. And in the presence of D-CPPene, when the rats were only able to encode and retrieve one experience, that is all they retrieved and we observed no decay over a three to five hour period, from the time of the initial encoding or replay of track 1 to the end of the final sleep session. To truly examine whether replay decays with time, we would first look at the replay of once experience over a long period of time. We would have the animals run on track 1 for a period of thirty minutes or run for 20 laps and then record sleep for a long period of time. Ideally we would record overnight. The technical limitation, however, would be that it is very difficult to keep stable tetrode recordings for long periods of time, as there can be minor movements in the tetrodes that would cause the tracking of clusters, or cells, to be lost. I have done recordings overnight, and find clusters can be tracked. However, in order to end up with a high number of stable cells, one must be able to start with a high number of isolated units.

To examine the competition effect of multiple experiences, we would have animals experience three tracks, but this time without a sleep 2 session in between tracks, just one final sleep that would last as long as recordings were stable. It is important to note that in addition to acquiring single unit activity, and local field potentials, we would also try and acquire an electromyogram (EMG). The EMG records electrical activity produced by skeletal muscles and helps one differentiate awake behavior state from sleep state. If we are to address consolidation of memories in the future it is important to know

what behavioral state the animal is in, as the process of memory consolidation may be different during different behavioral states.

Concluding Remarks

This has dissertation examined the role of neuronal and molecular mechanisms associated with synaptic plasticity in the generation of replay sequences in the hippocampus. Our data supports the theory that NMDAR activation is required for replay, however a subtle distinction was found regarding this requirement. The first time an animal experiences an environment, NMDAR activation is required in order for replay to be observed either during that experience, or subsequently. However, if an environment has already been experienced, so that plastic changes have already occurred, then replay sequences may be observed even when further NMDAR-dependent plasticity is being blocked. Thus, rather than the generation of replay being dependent on NMDAR function, it appears to be only the initial encoding of experience into memory that requires NMDAR dependent plasticity, in order that these encoded memories may be subsequently replayed.

In addition to multiple reports that place fields are still observed under blockade of NMDARs (Ekstrom et al., 2001; Kentros et al., 1998; McHugh et al., 1996; Nakashiba et al., 2008; Nakazawa et al., 2002; Suh et al., 2011), which we have replicated here, it has further been demonstrated that disruption of replay through electrical stimulation coincident with detected SWR events leads to impaired memory, while place field responses are completely spared(Jadhav et al., 2012). Thus, SWR-associated activity must perform a memory-related function beyond that provided by place field responses alone. We further examined decoded position and found that an animal's location could be decoded accurately even when using the larger and sparser place fields observed under

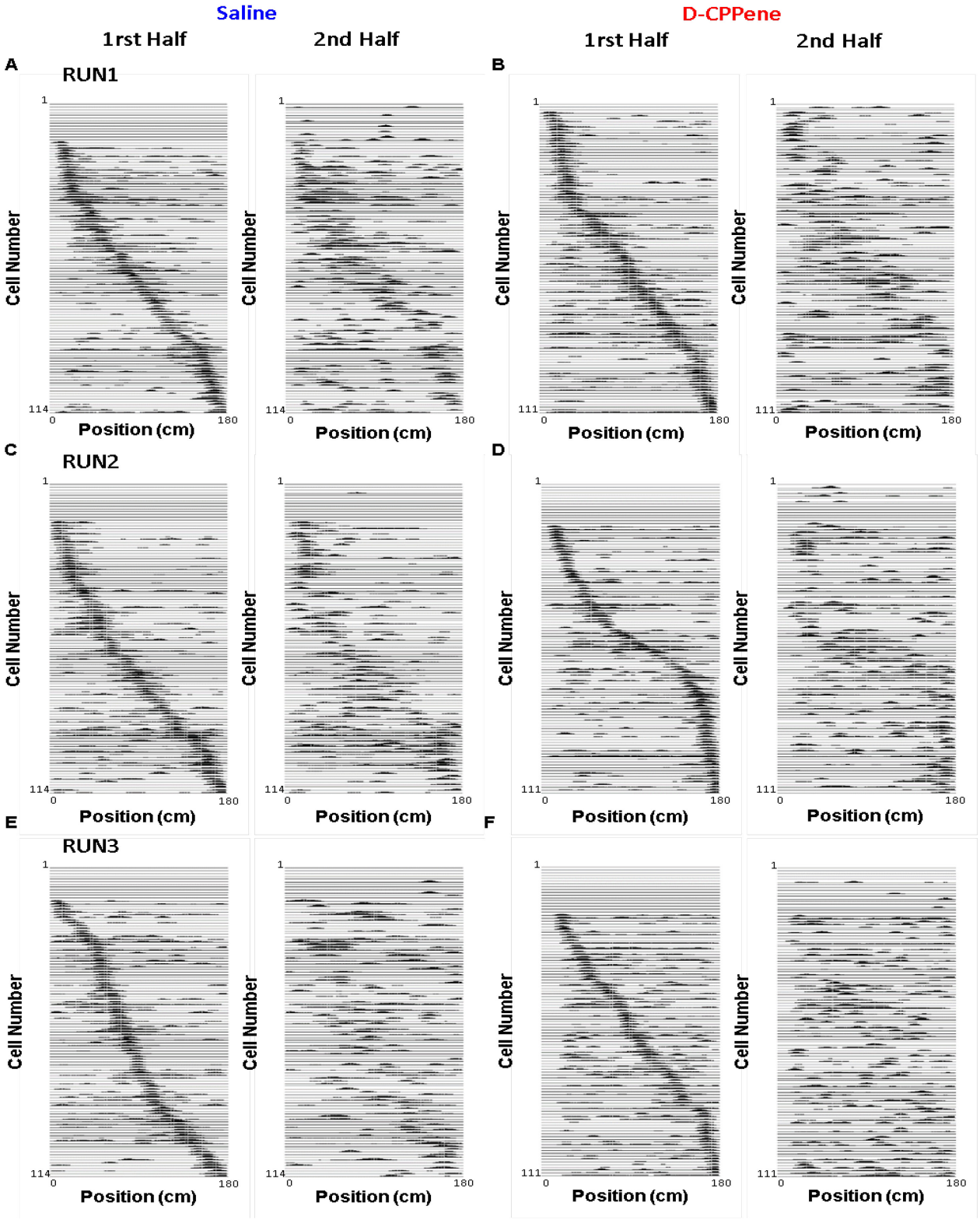
NMDAR blockade. It has been reported that decoding accuracy increases with the number of cells involved(Wilson and McNaughton, 1993). Given the fact that tens of thousands of CA1 place cells are available to the animal to decode each potential location in an environment, it is likely that errors in position estimation due to sparser fields are negligible.

Our distinction between encoding and retrieval maps onto corresponding distinctions at two different levels of analysis. First, it has long been known that blockade of hippocampal CA1 NMDARs has been shown to have little effect on AMPAR-mediated synaptic transmission (Collingridge et al., 1983), and therefore it is reasonable to conclude that place cells may participate in the replay of established memories using spared mechanisms of synaptic transmission (Martin et al., 2000; Martin and Morris, 2002). Second, it is equally long established that hippocampally dependent memory requires NMDAR function during encoding but not during retrieval (Bannerman et al., 1995; Caramanos and Shapiro, 1994; Morris, 1989; Morris et al., 1986; Saucier and Cain, 1995; Shapiro and Caramanos, 1990; Steele and Morris, 1999). Therefore, our result may offer a way to bridge these two results. Given that the mnemonic role of the hippocampus is presumably mediated by hippocampal neurons, we may hypothesize that replay provides the memory retrieval mechanism whereby memories encoded in synaptic networks are reactivated, using a process that does not itself require NMDAR function. The hypothesis that replay equates to memory retrieval is further supported by recent observations that hippocampal replay sequences can occur on-demand during a memory task, and provide task-relevant information about the route to a remembered goal(Pfeiffer and Foster, 2013).

It has been demonstrated that, provided the logical contingencies between locations in an environment are known or have at least been sampled, the hippocampus is capable of generating place-cell sequences in an order that has never been experienced(Davidson et al., 2009; Diba and Buzsaki, 2007; Foster and Wilson, 2006; Gupta et al., 2010; Karlsson and Frank, 2009), and sequences that depict as yet never experienced trajectories(Gupta et al., 2010; Pfeiffer and Foster, 2013), in particular, novel paths to a remembered goal(Pfeiffer and Foster, 2013). Together these results raise the possibility that the role of NMDAR dependent plasticity may not consist of recording or reinforcing specific sequences of experience for veridical playback(Morris et al., 2003; Redish and Touretzky, 1998), but rather support the learning of underlying contingencies in environments. In this sense, replay sequences might be thought of as constructive attempts to read out information from a learned cognitive map(O'Keefe and Nadel, 1978), where the underlying contingencies are learned from experience and stored in the synaptic matrix, but where specific retrieval events can be modified uniquely to suit the requirements of the current task. In this way, hippocampal replay provides a model system that can reconcile the mnemonic (Scoville and Milner, 1957) and constructive (Hassabis et al., 2007) aspects of the brain's episodic memory system(Tulving, 2002).

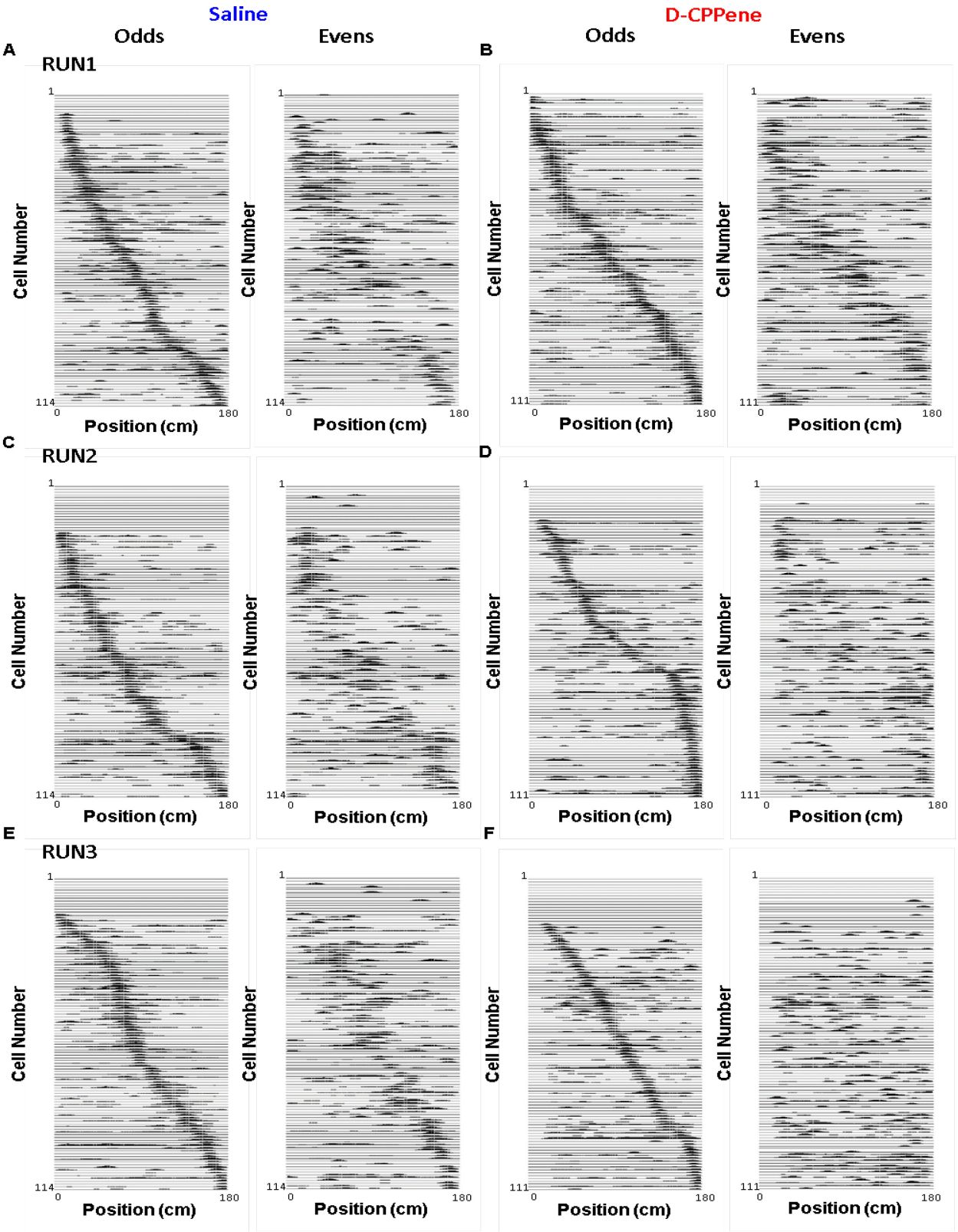
It is our hope that this dissertation and the experimental work within it serves to inform future studies of the functional role of hippocampal networks in learning and memory. It is a very exciting time as the development of neurophysiological techniques continues to advance and is combined with proven molecular and transgenic approaches, data which was once only imaginable will be acquired and further enhance the search for the "engram".

Appendix A


Saline
D-CPPene
1rst Half
2nd Half
1rst Half
2nd Half
A
RUN1
B
C
RUN2
D
E
RUN3
F
Cell Number
Position (cm)
1
114
111
0
180

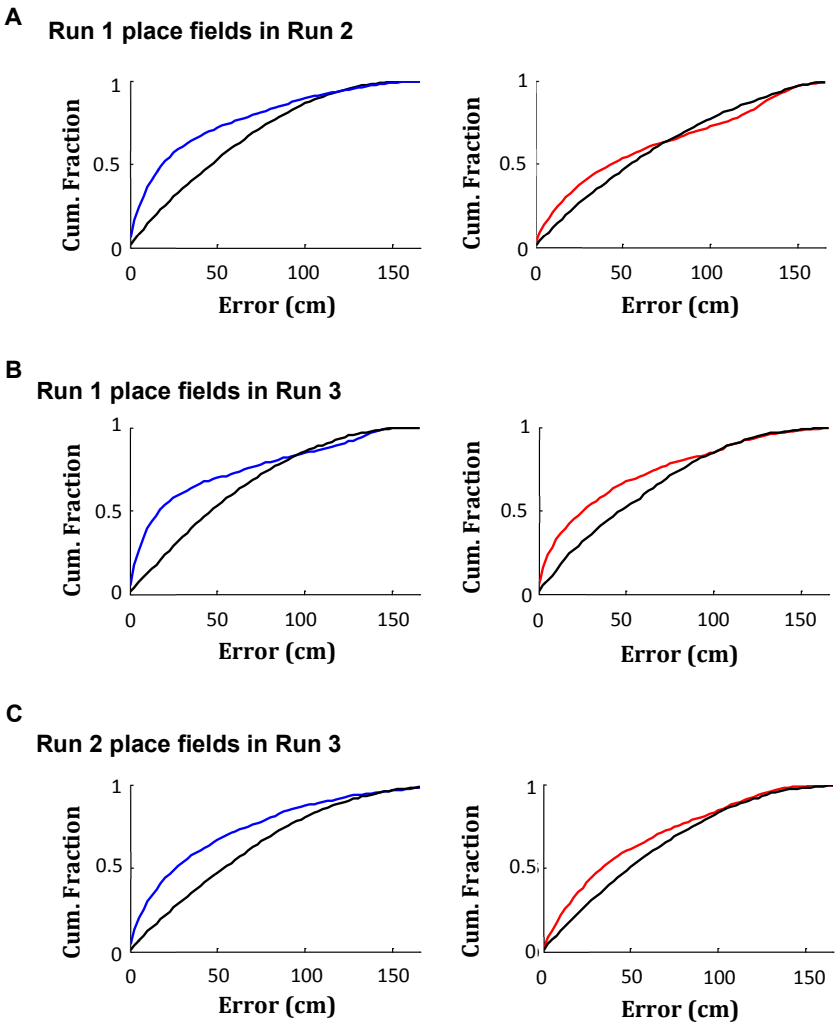
Appendix A. Place Field Maps Within Runs Across Different Laps. The same 114 place fields under saline and the same 111 place fields under D-CPPene, same animal separate recording days, are plotted formed after the first half (first ten laps) and the last half (last ten laps) of each running session.

Appendix B


Saline
D-CPPene
Odds
Evens
Odds
Evens
A
RUN1
B
C
RUN2
D
E
RUN3
F
Cell Number
Position (cm)
1
114
111
0
180

Appendix B. Place Field Rate Maps Within Runs Across Odd and Even Laps. Same as in Appendix A, but now looking at the odd and even laps of the same sessions across the three runs.

Appendix C

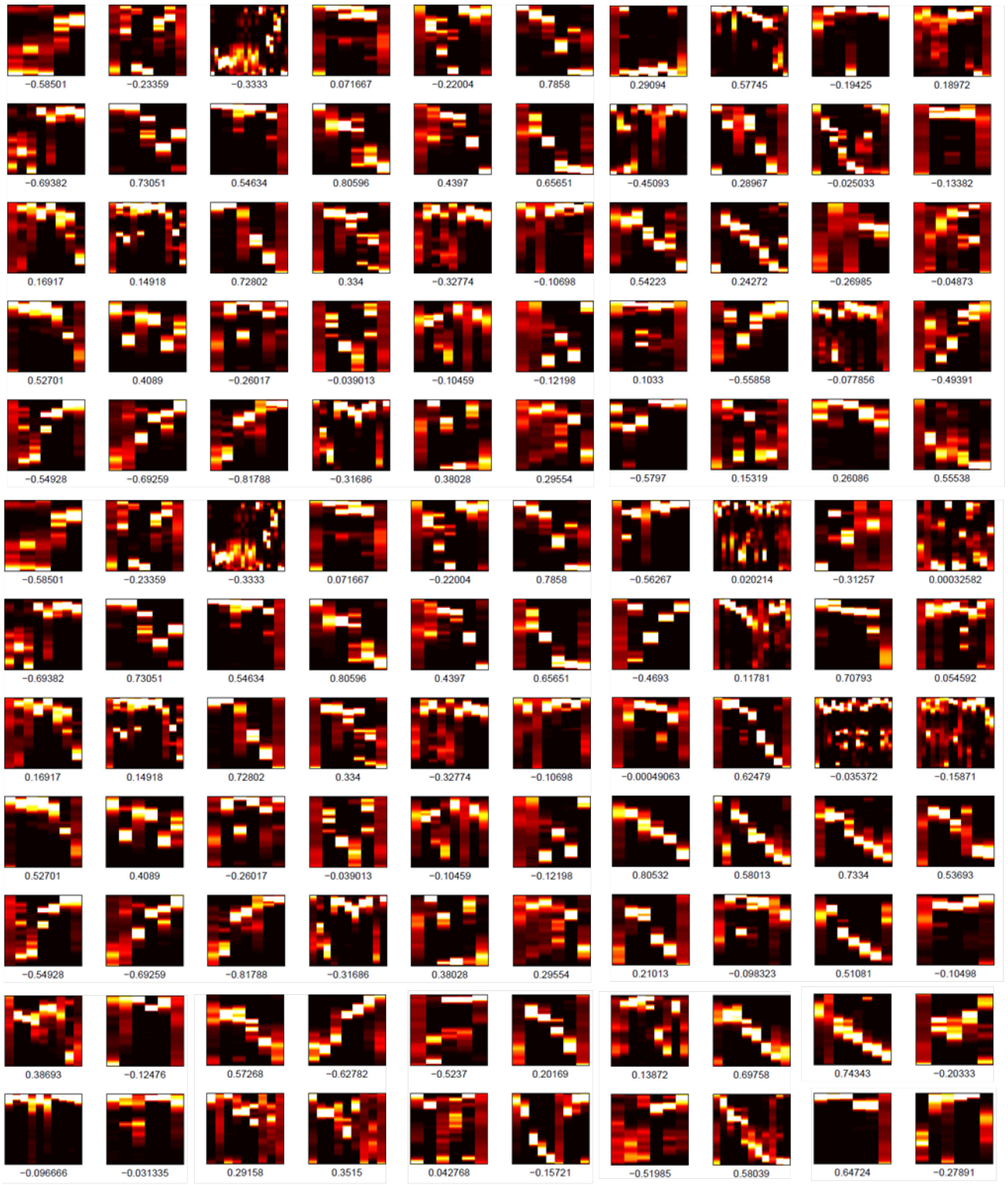


Appendix C. Cross-Track Decoding. Error measures for position estimation during each run using the place field positions of a previous run. Decoding of Run2 and Run3 using place fields from Run1, the and decoding of Run3 using place fields from Run2. Plotted is the group data for each run for both saline (in blue) and D-CPPene (in red) conditions.

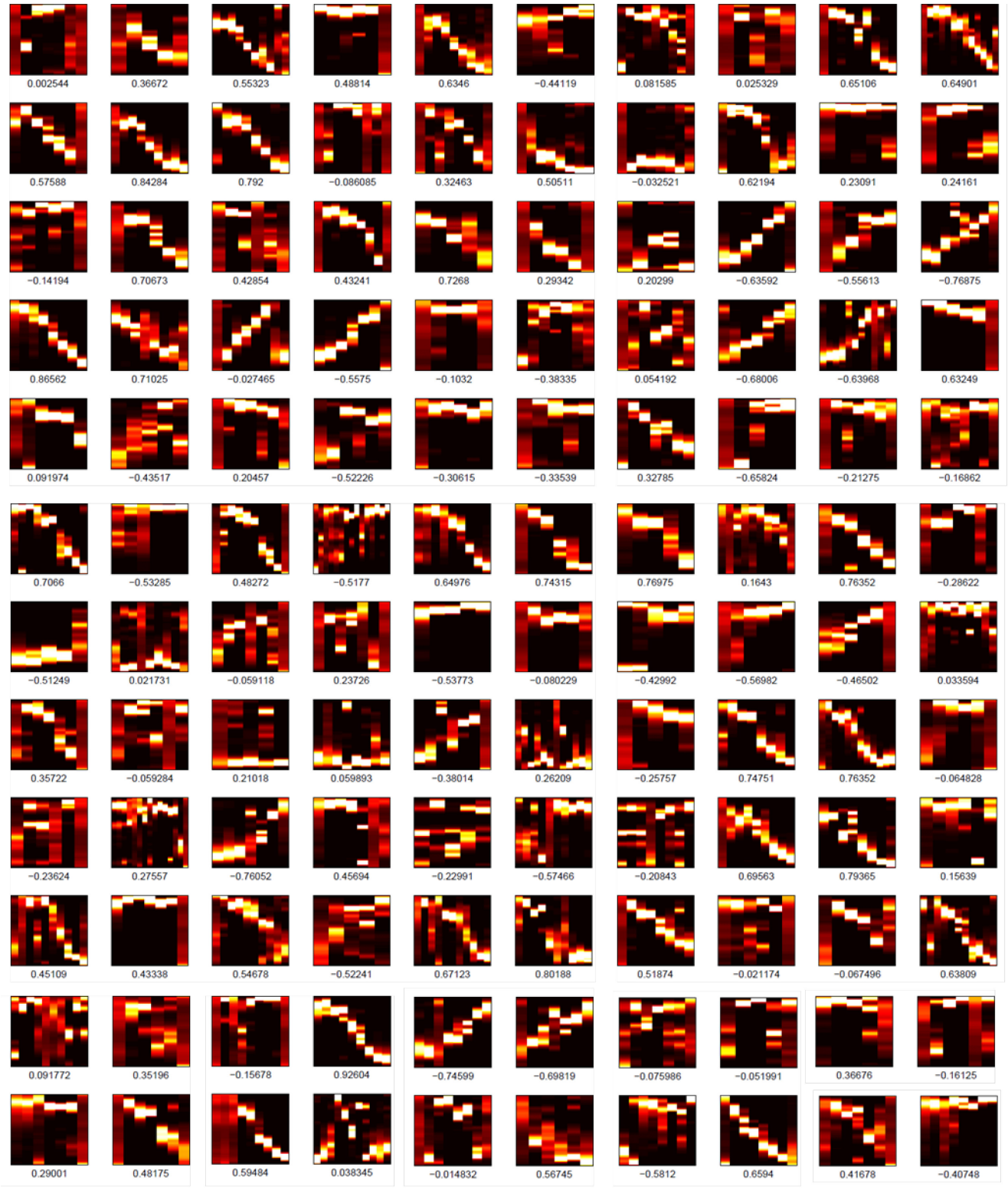
Appendix D

Appendix D. All Candidate Events for Saline Run2 (1 animal, 1 session). 397 candidate events for Run2.

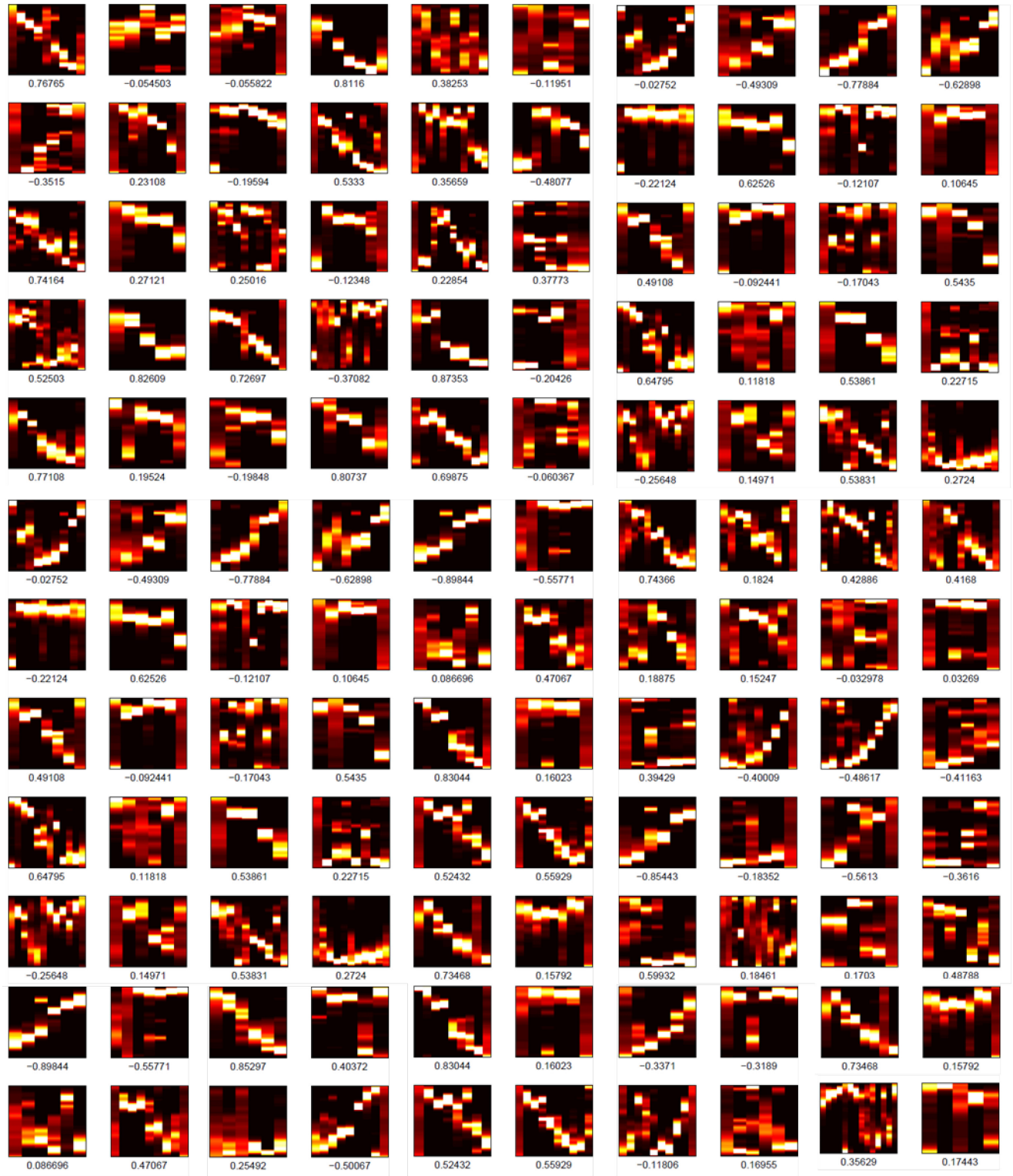
Candidate Events – Saline Run 2



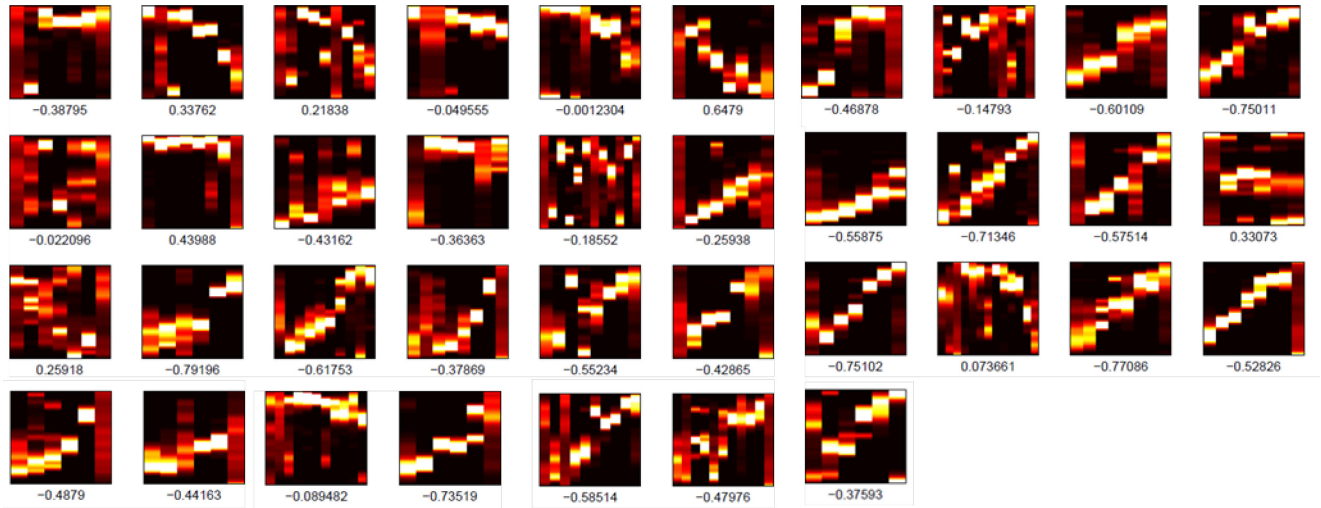
(Cand. Events 1-120)



(Cand. Events 120-240)



(Cand. Events 241-360)

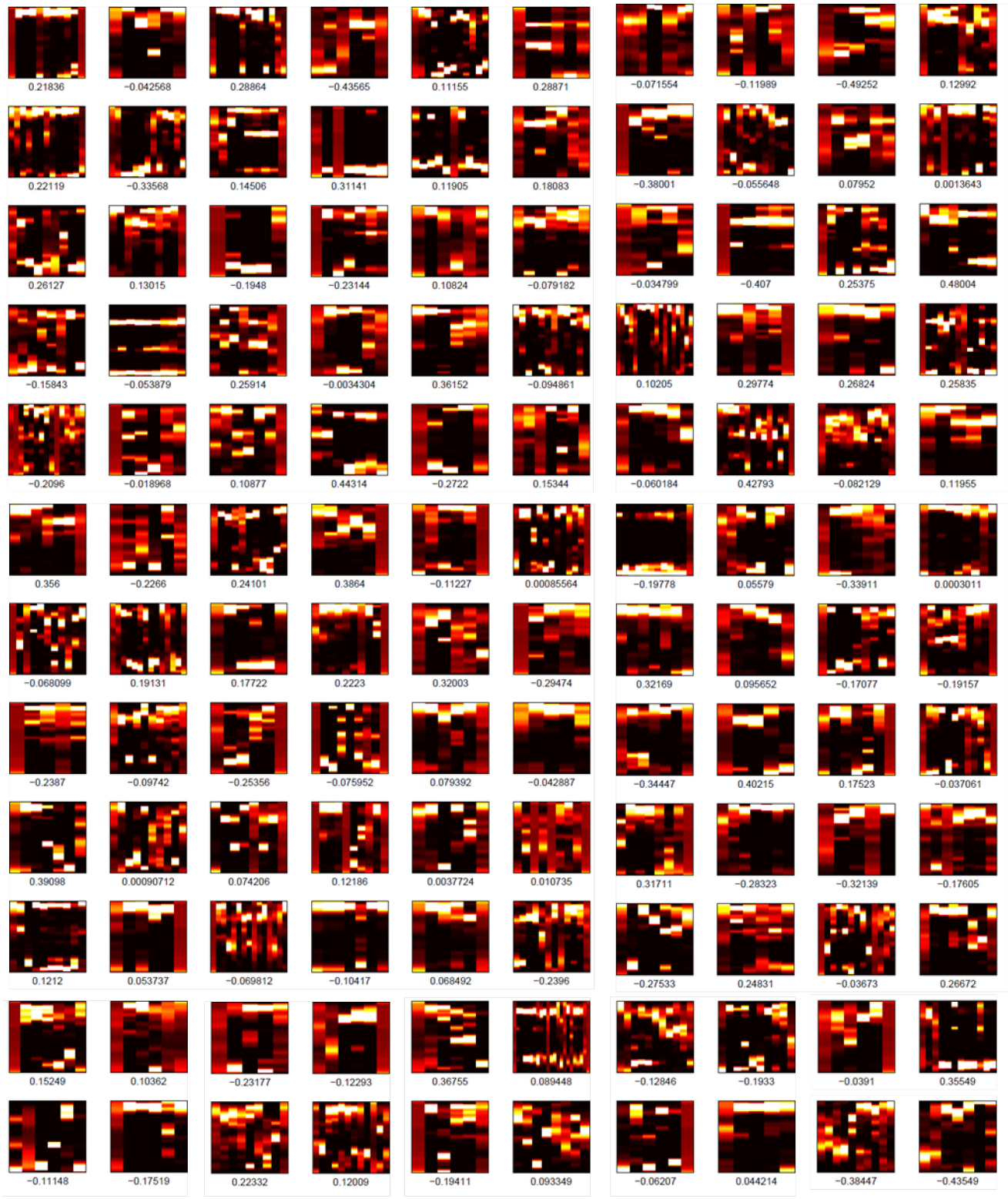


(Cand. Events 360-397)

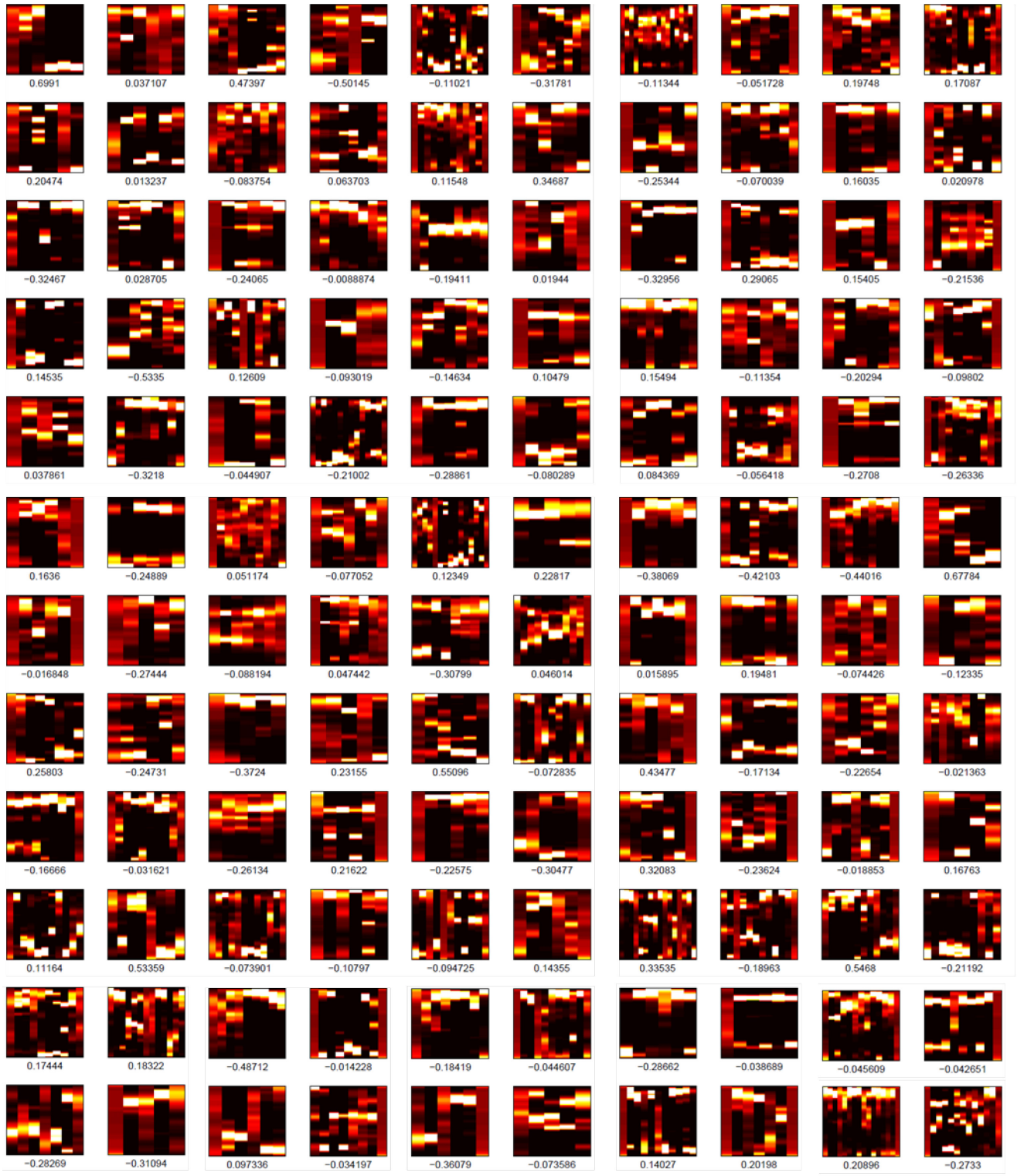
Appendix E

Appendix E: All Candidate Events for D-CPPene Run2 (1animal, 1session). 293 candidate events for Run2.

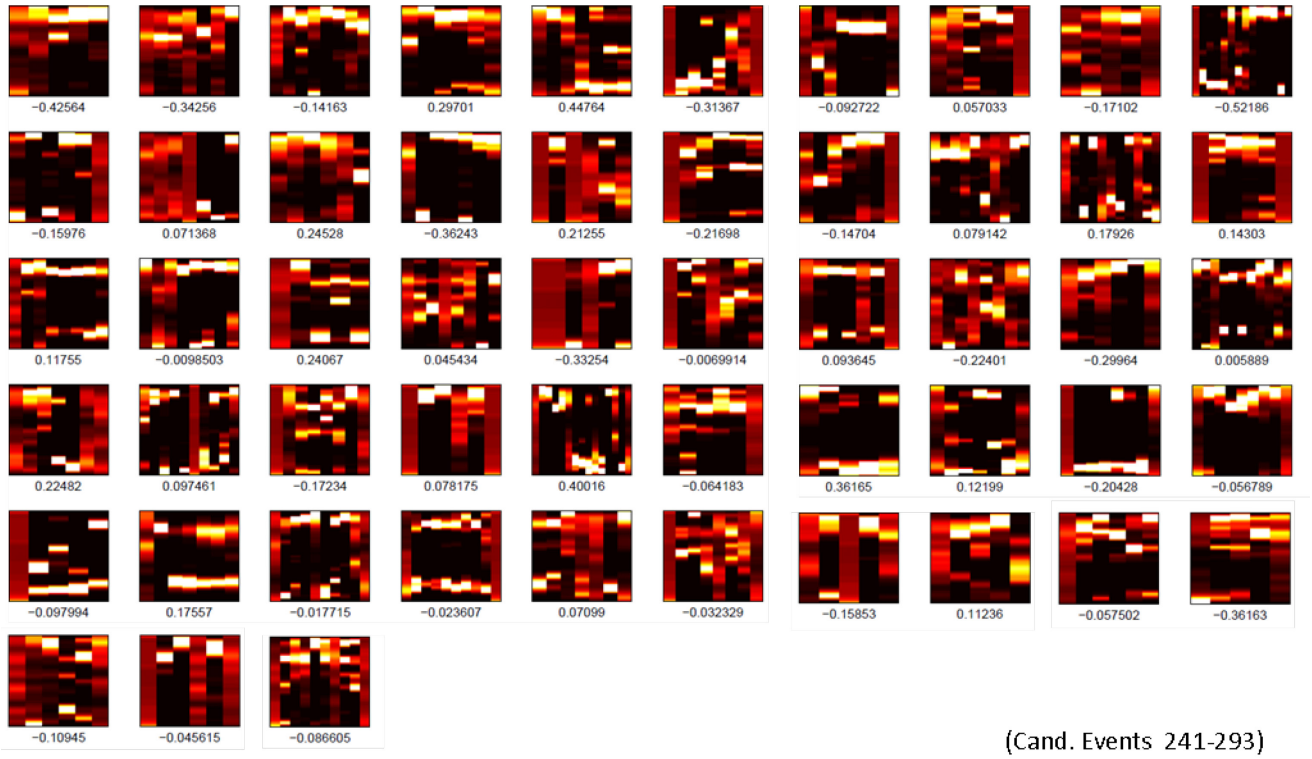
Candidate Events – D-CPPene Run 2



(Cand. Events 1-120)

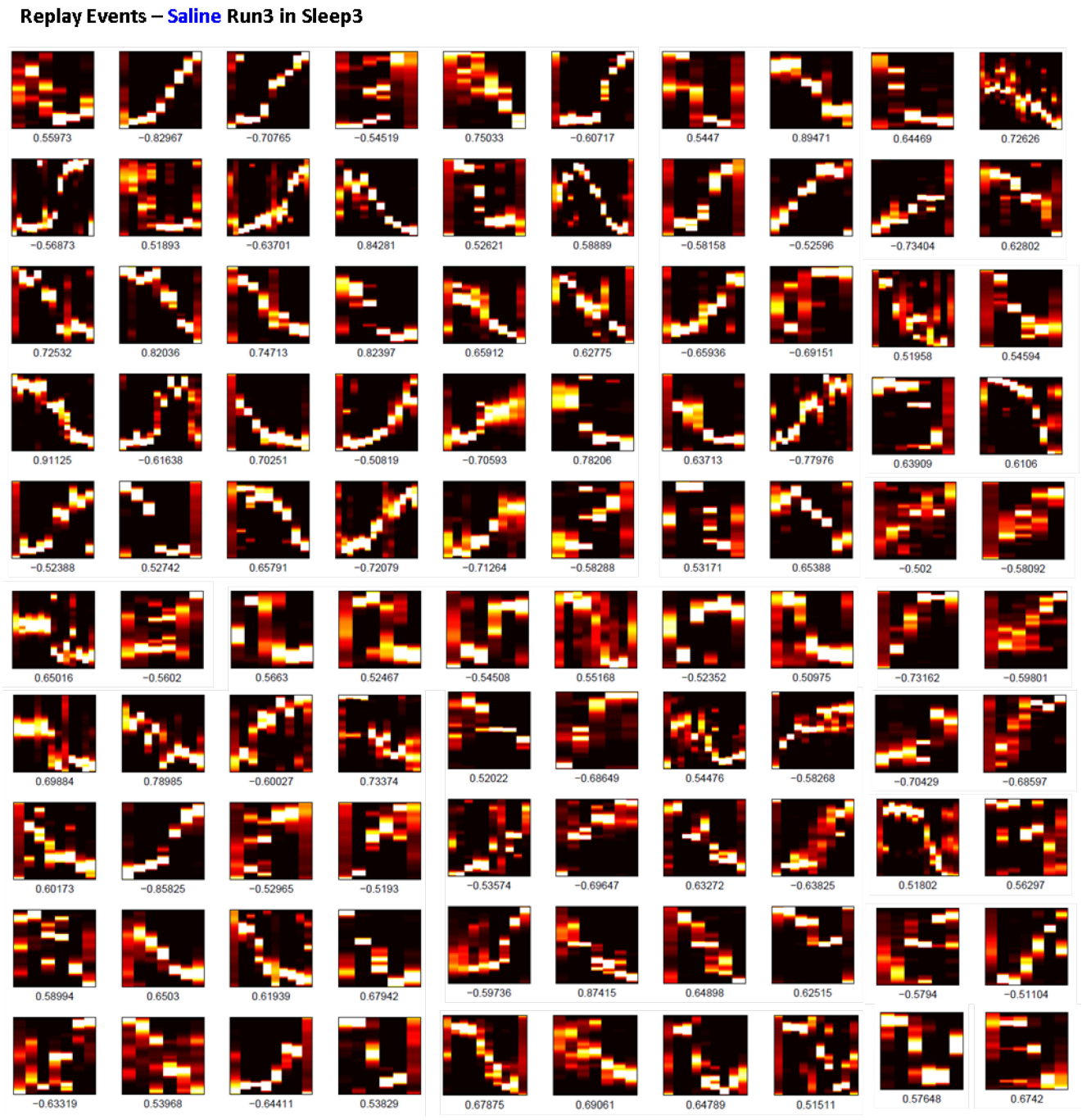


(Cand. Events 121-240)



(Cand. Events 241-293)

Appendix F

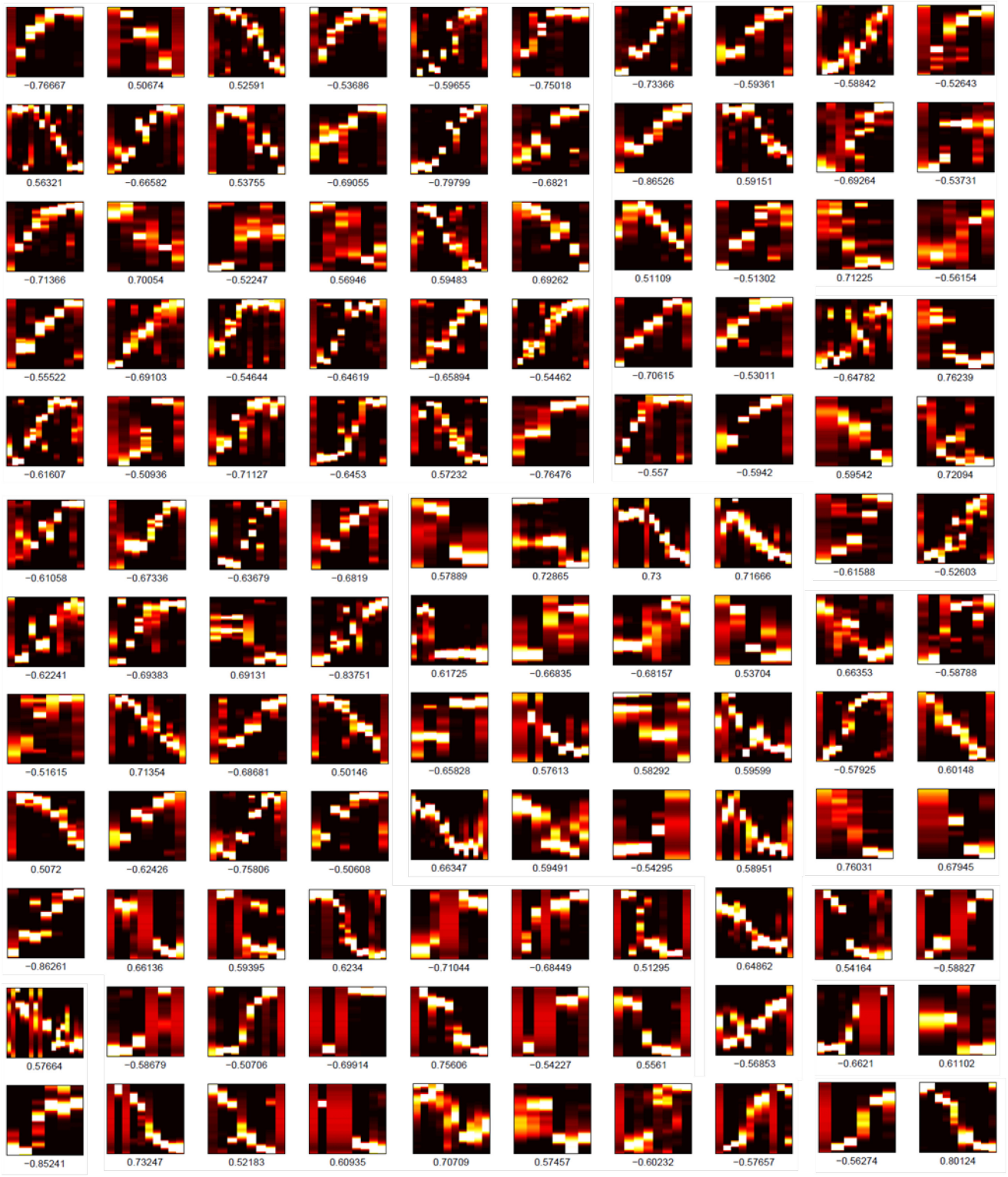


Appendix F: Retrieved Replays of Run 3 in Final Sleep (Sleep3) in Saline Condition (1 animal, 1 session).

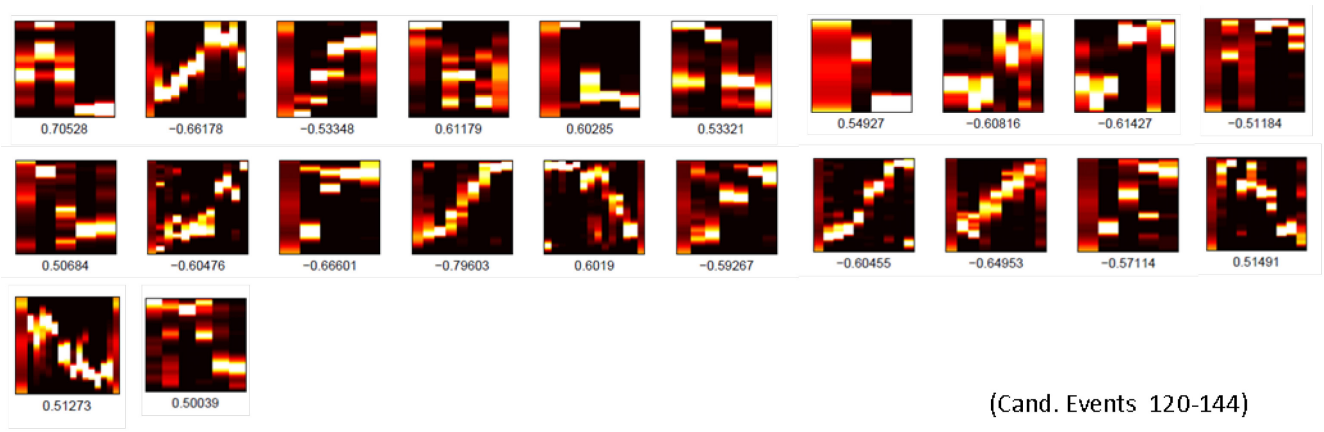
Appendix G

Appendix G: Retrieved Replays of Run1 in Final Sleep (Sleep3) under NMDAR Antagonism (1 animal, 1 session)

Replay Events –D-CPPene Run1 in Sleep3

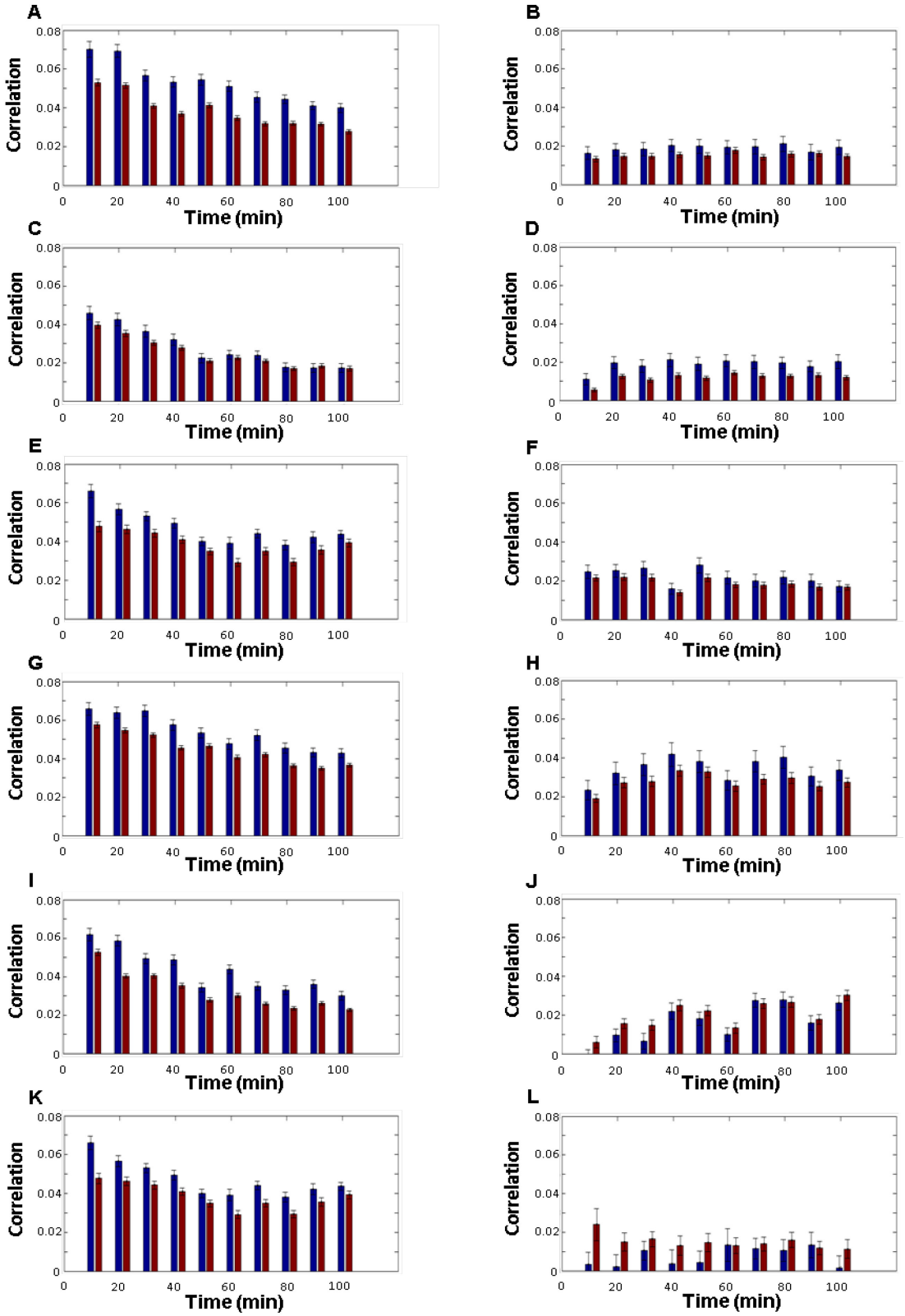


(Cand. Events 1-120)



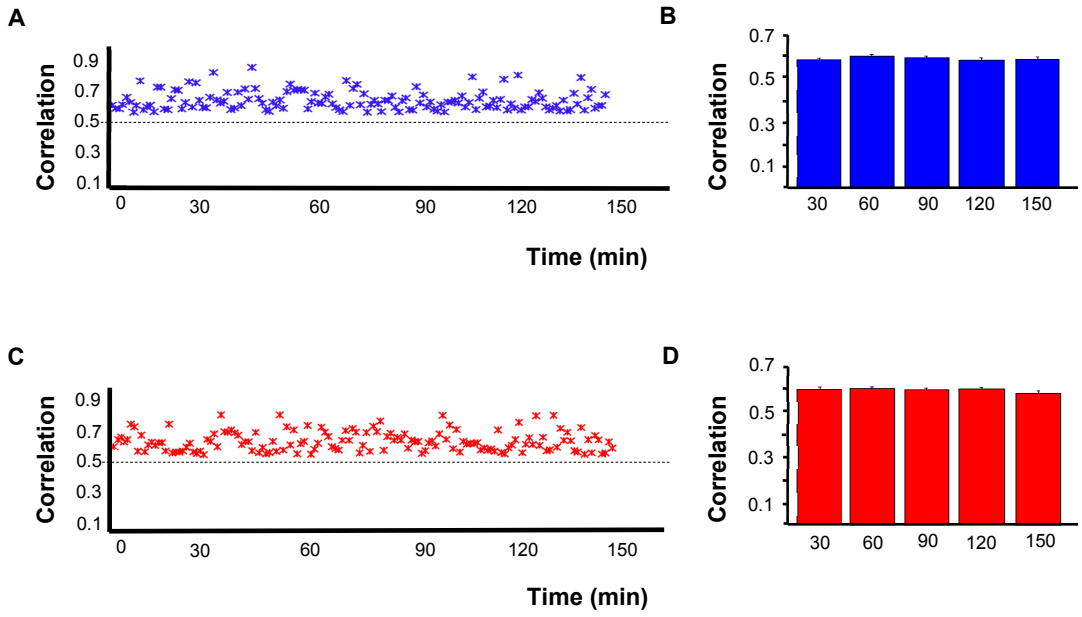
(Cand. Events 120-144)

Appendix H


A
B
C
D
E
F
G
H
I
J
K
L
Correlation
Time (min)
0
0.02
0.04
0.06
0.08
0
20
40
60
80
100

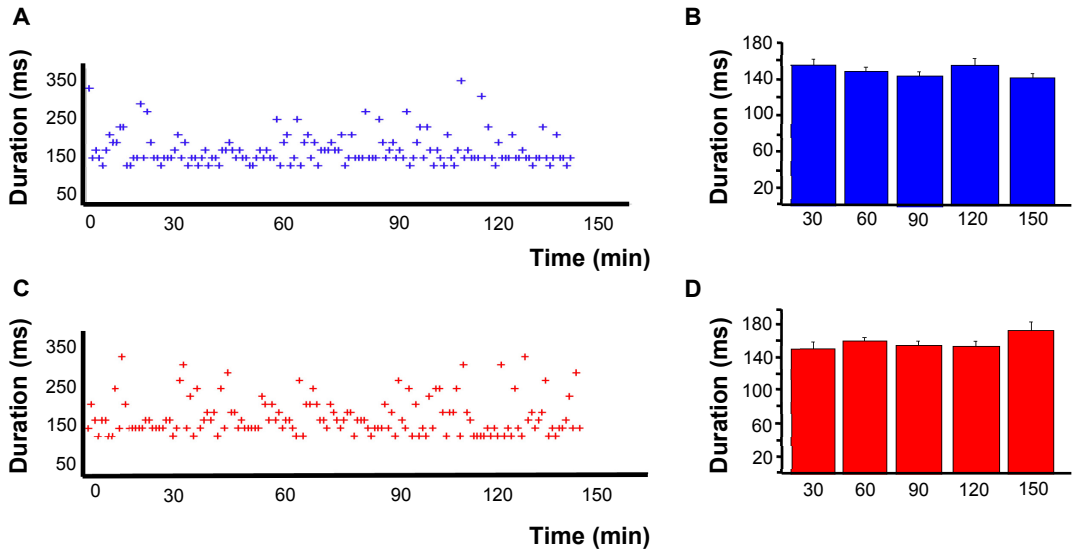
Appendix H. Pairwise Analysis of Decay of Retrieved Replays in Final Sleep. Pair wise correlations every 10 minutes in the first 100 minutes of sleep 3 are plotted. All six sessions of Sleep 3 are plotted for both conditions. The right column (B, D, F, H, J, and L) are all the sessions under D-CPPene and pairwise activity reflects reactivation of Run3 in Sleep3. The left column (A, C, E, G, I and K) are all the sessions under saline.

Appendix I



Appendix I. Correlation of Retrieved Replays Over Time. A and C, Example of correlations for all replays for one session for both conditions throughout the entirety of sleep3. B and D, Bar graphs with averages of replay correlation for group data every 30 min. For saline, replays of run 3 in sleep3 are used. For D-CPPene, replays of run1 in sleep3 are used.

Appendix J



Appendix J. Duration of Retrieved Replays Over Time. A and C, Example of durations for all replays for one session for both conditions throughout the entirety of sleep3. B and D, Bar graph with averages of replay correlation for group data every 30 min. For saline, replays of run 3 in sleep3 are used. For D-CPPene, replays of run1 in sleep3 are used.

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Post-Bac Research	2005-2007	Alex Kolokin, NIH
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Publications:

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Silva D, Dikkes P, Barnes M, Lopez MF. Decreased Motor Neuron Survival in Igf2 Null Mice After Sciatic Nerve Transection. Neuroreport, 20(16):1414-8, 2009.

Silva D, Venihaki M, Guo WH, Lopez, MF. Igf2 Deficiency Results in Delayed Lung Development at the End of Gestation. Endocrinology, 147:5588-5591, 2006.

Posters and Abstracts:

D Silva, T Feng, DJ Foster. Dissociation between encoding and retrieval in the molecular mechanisms mediating hippocampal replay. Johns Hopkins Univ. Sch. Of Med., Baltimore MD (Neuroscience 2012, currently writing manuscript)

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